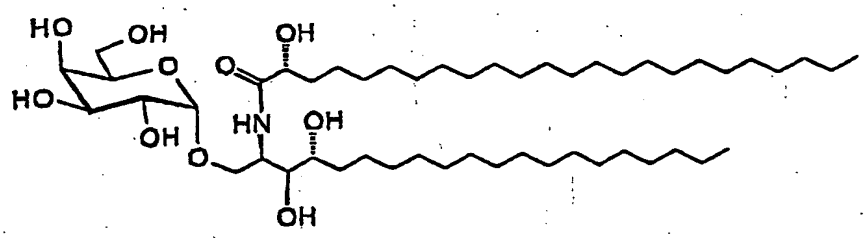
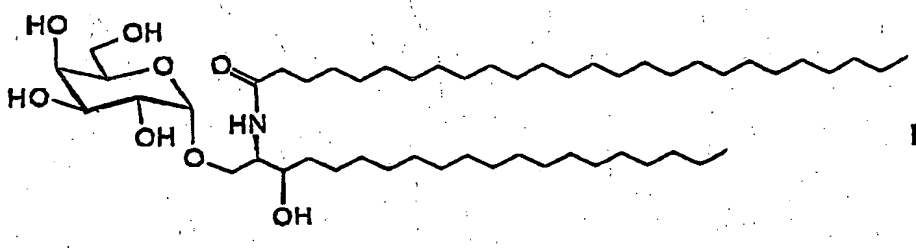


AGL-9b

Agelaspin-9b (**AGL-9b**) was isolated from marine sponge, *Agelas mauritianus*, and showed antitumor activity against melanoma.

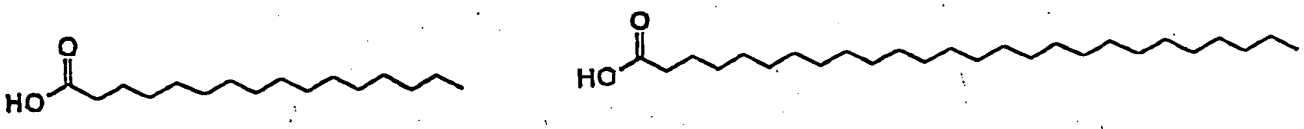


KRN7000

KRN7000 is a synthetic analog of AGL-9b and is currently being evaluated as antitumor and immunomodulating agent in the clinic.

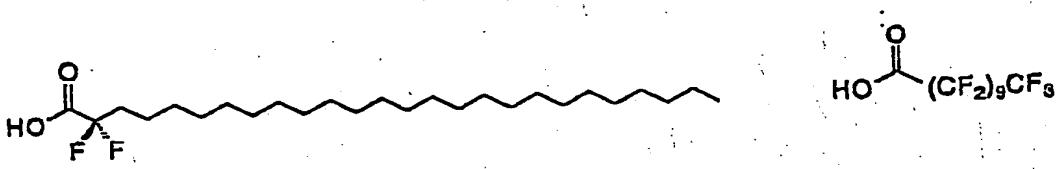
FIG. 1 α-GalCer from natural sources and chemical synthesis as potential immunotherapeutics

Saturated fatty acid:

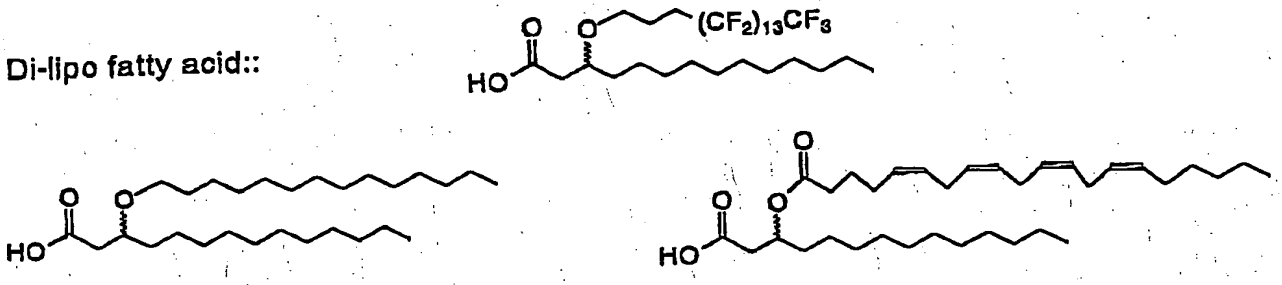


Unsaturated fatty acid:

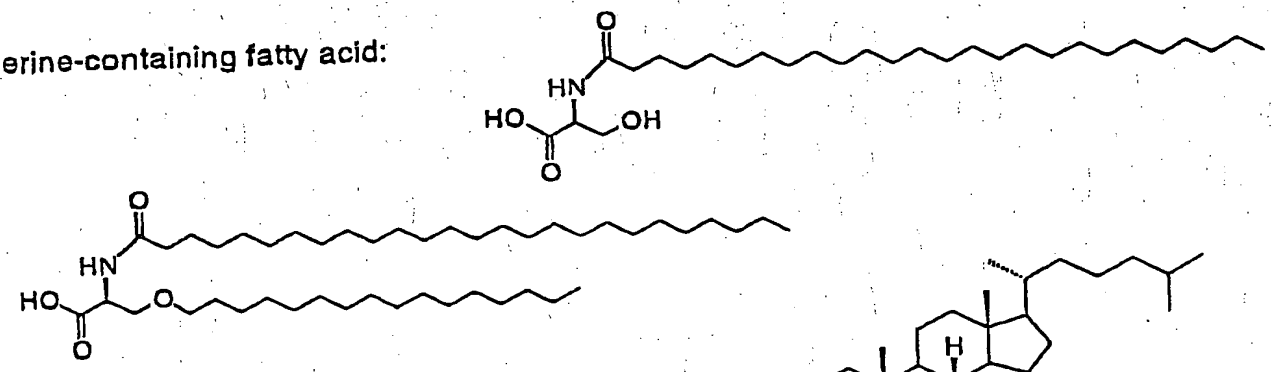
Fluoro-substituted fatty acid::



Di-lipo fatty acid::



Serine-containing fatty acid:



Steroid-derived lipo acid:

FIG. 2 Structures of fatty acids used in the design of α-GalCer mimics.

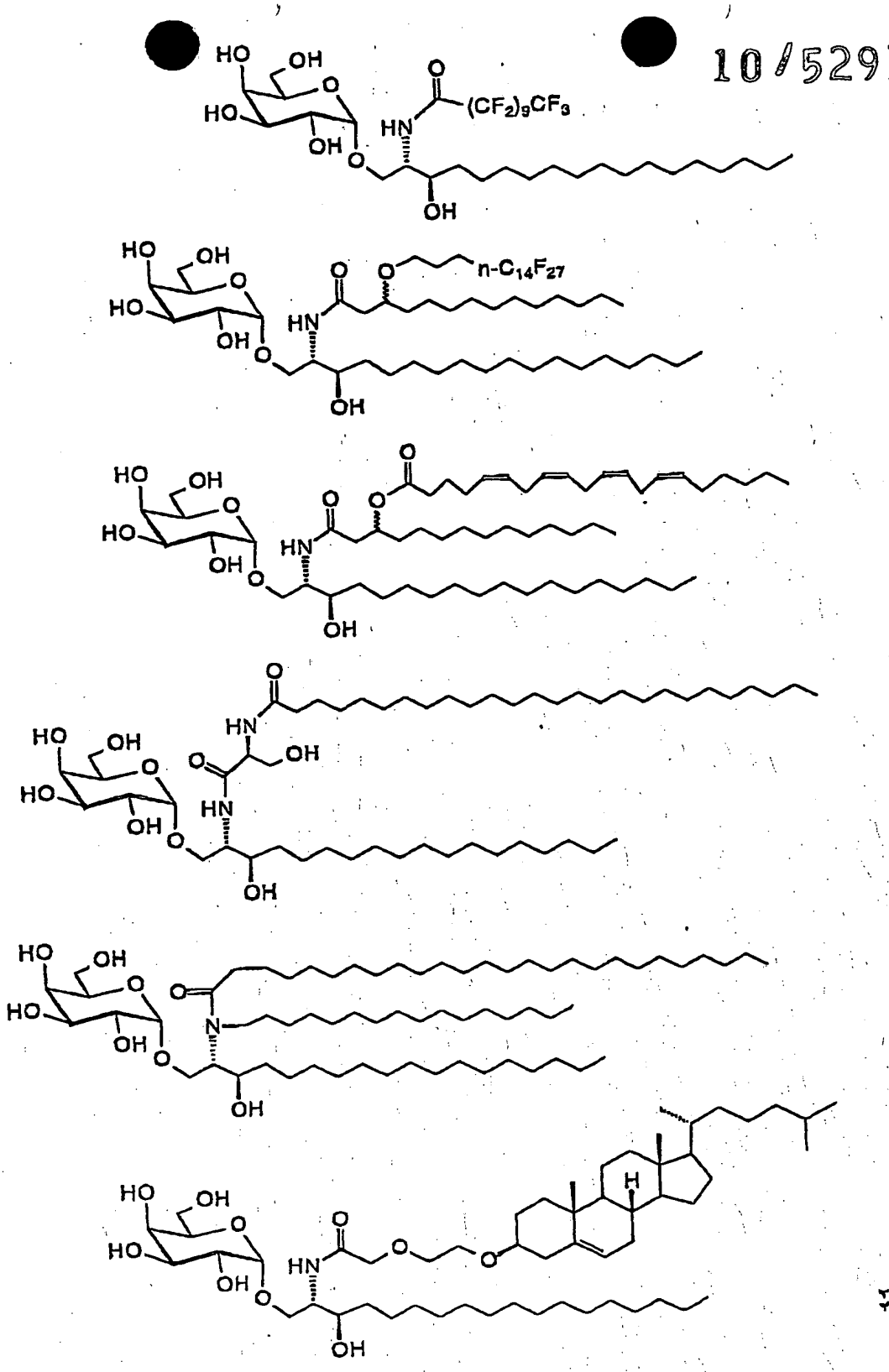


FIG. 3 α-GalCer analogues with modified *N*-acyl group on sphingosine

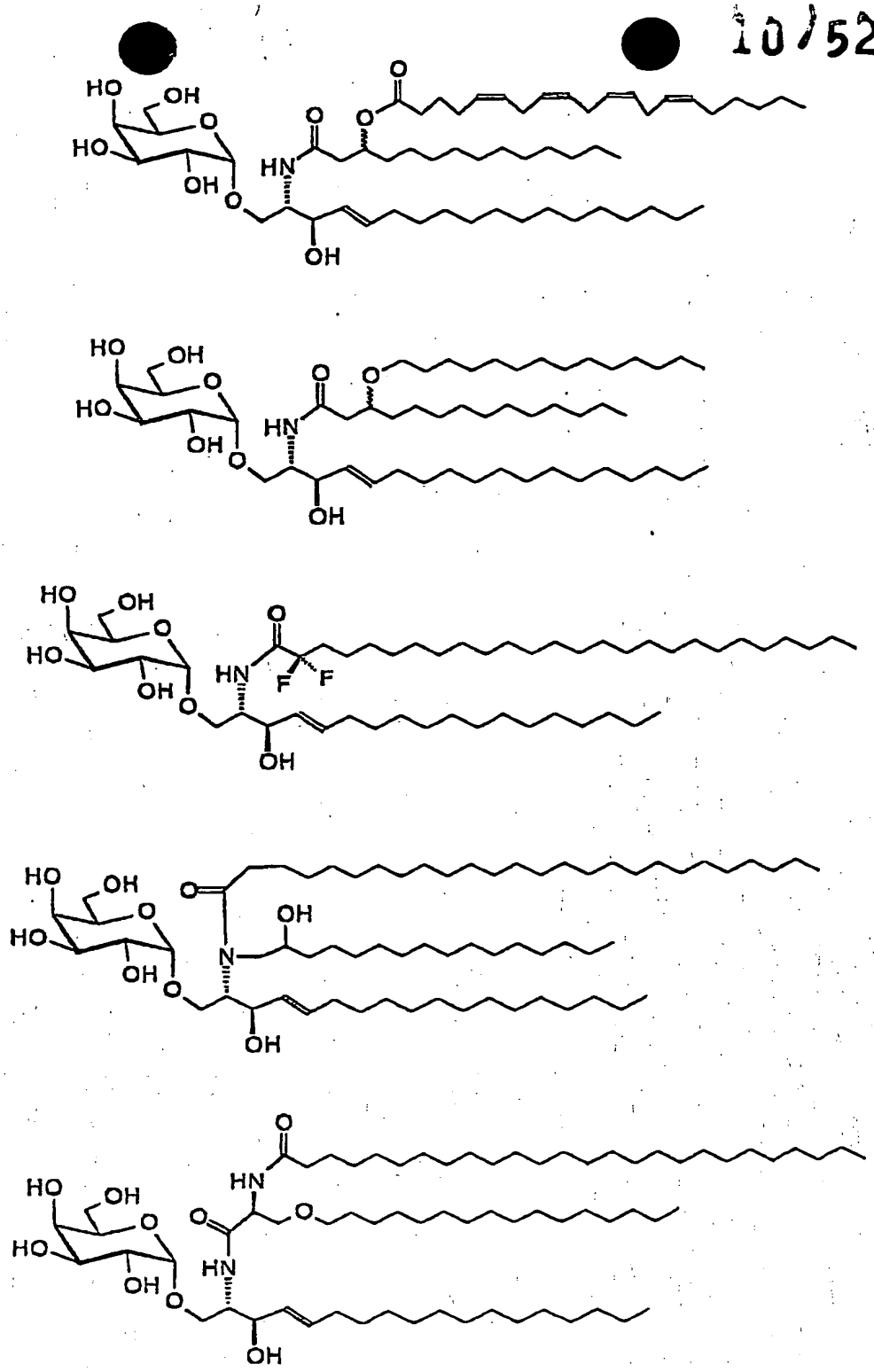


FIG. 4 α-GalCer analogues with *E*-4,5-ene-sphingosine and modified *N*-acyl groups

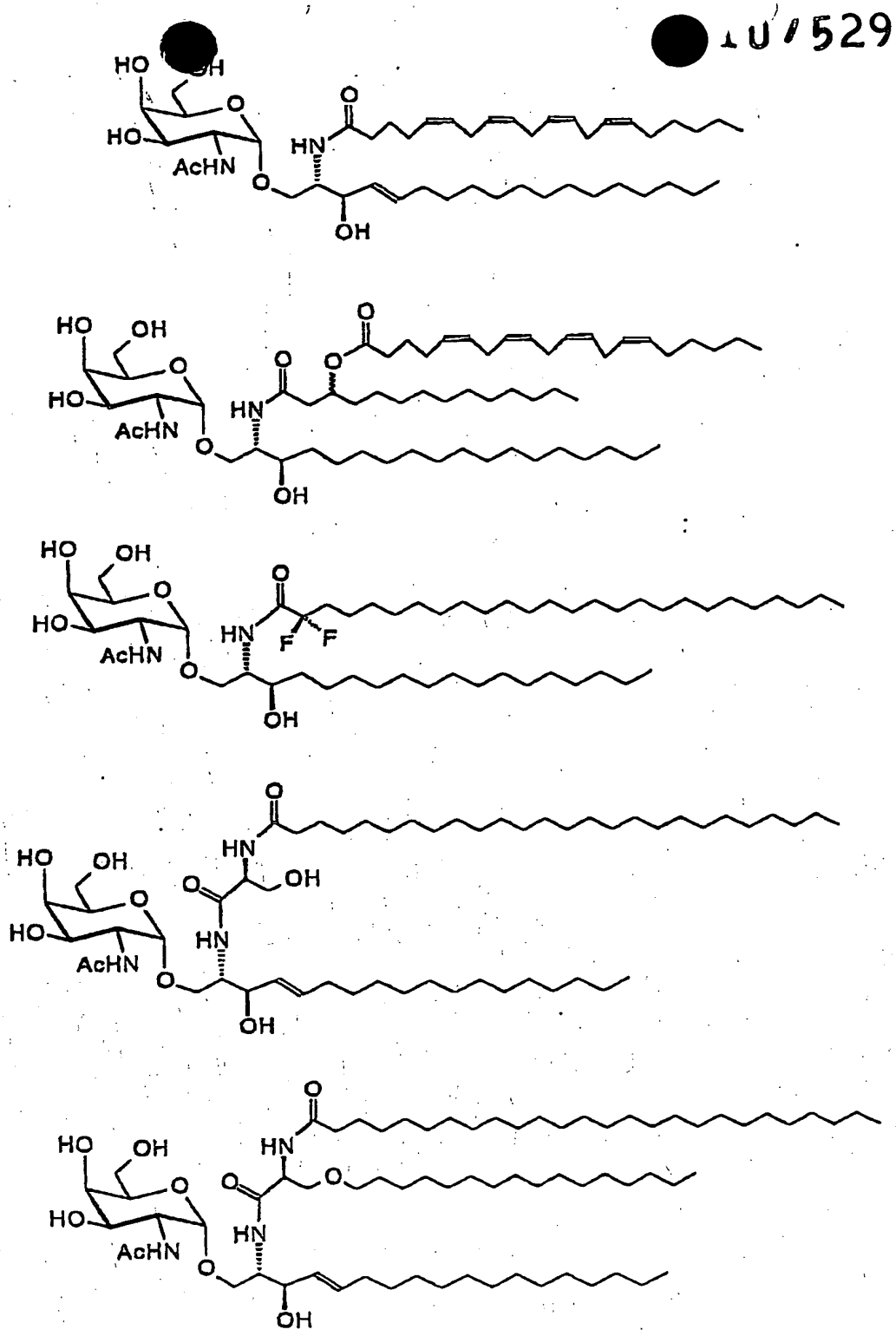


FIG. 5 α-GalCer analogues with GalNAc α-linked to sphingosine carrying modified *N*-acyl groups

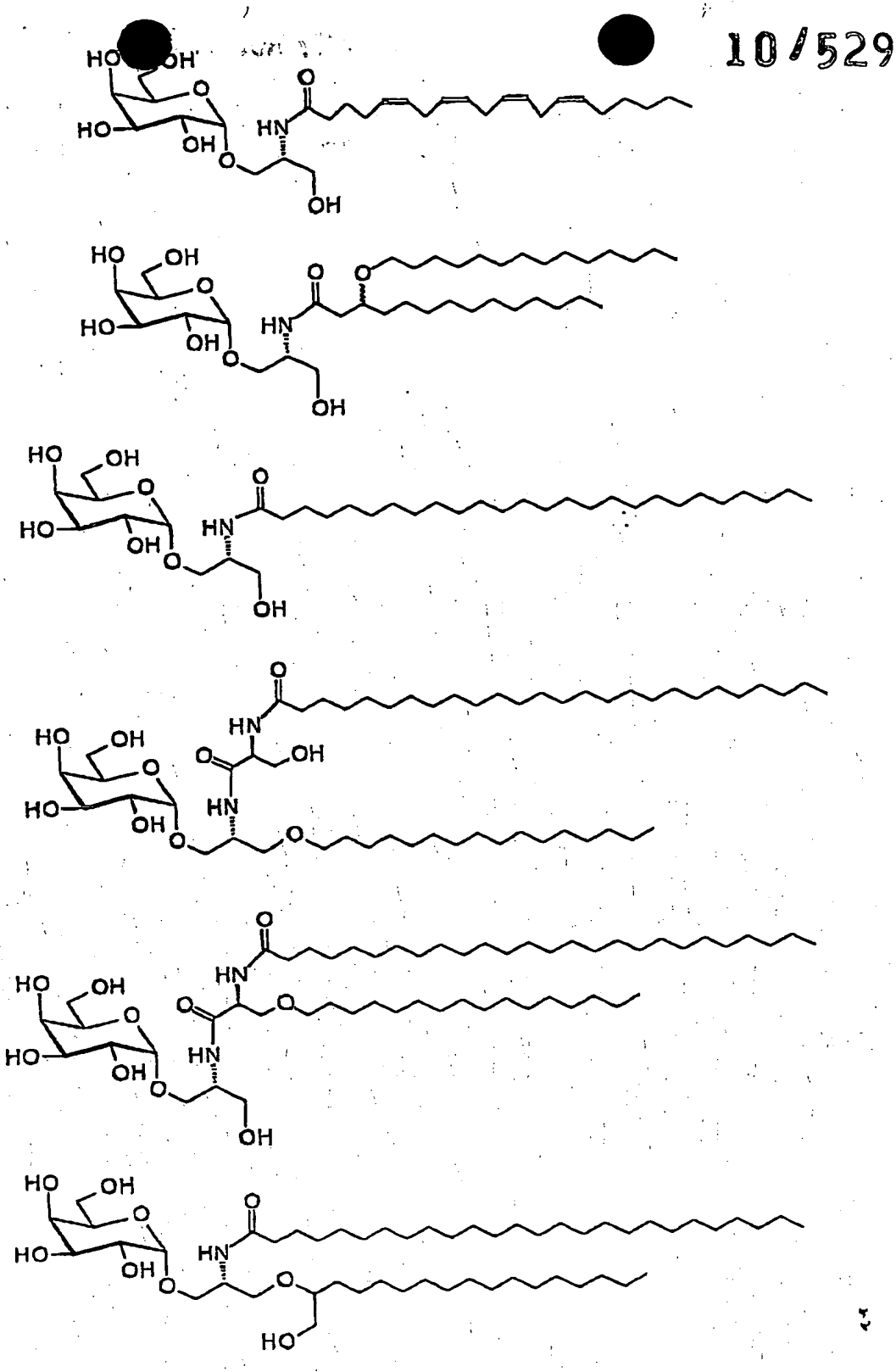


FIG. 6 α-GalCer analogues based on serinol

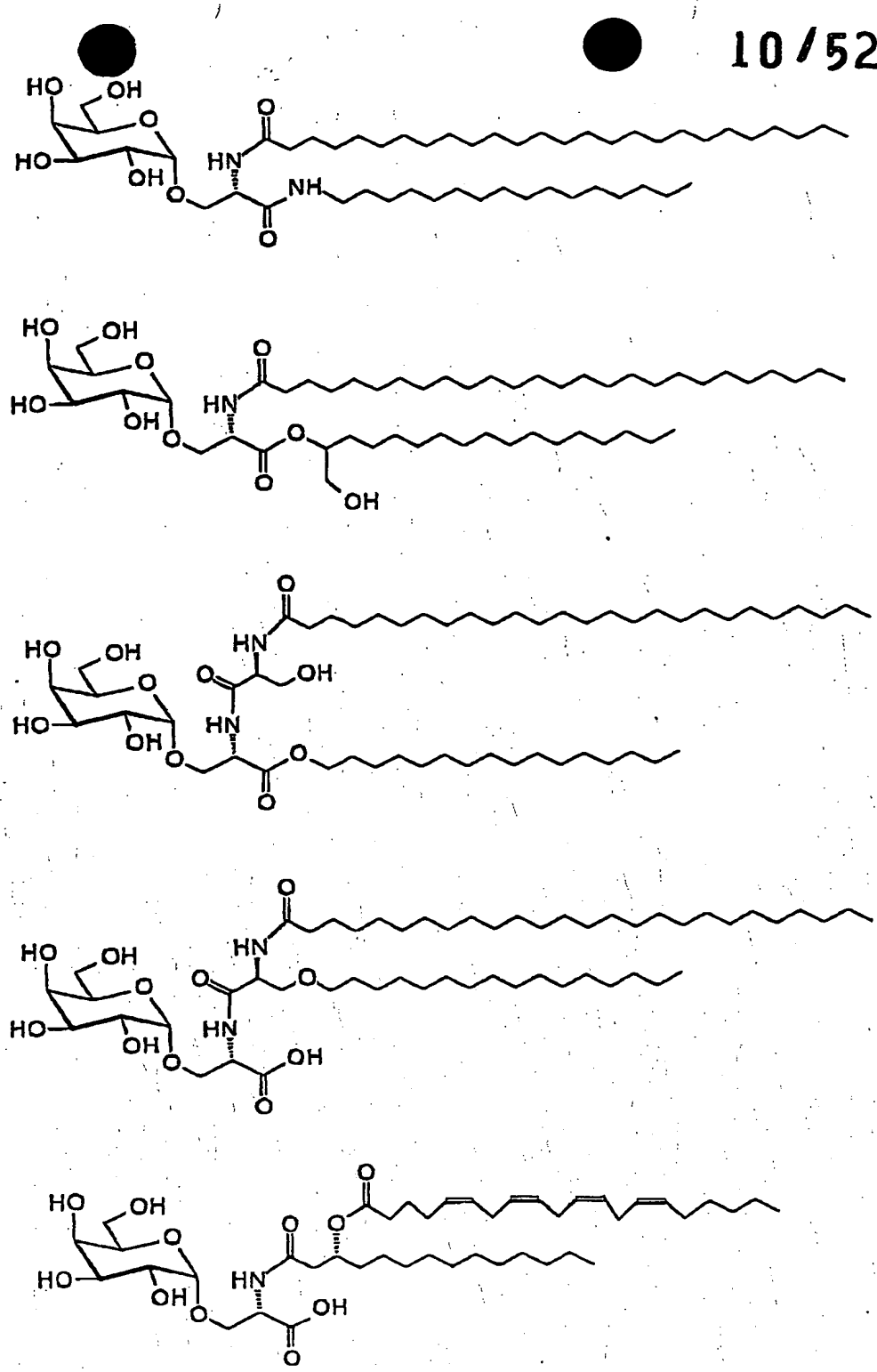


FIG. 7 α-GalCer analogues based on serine

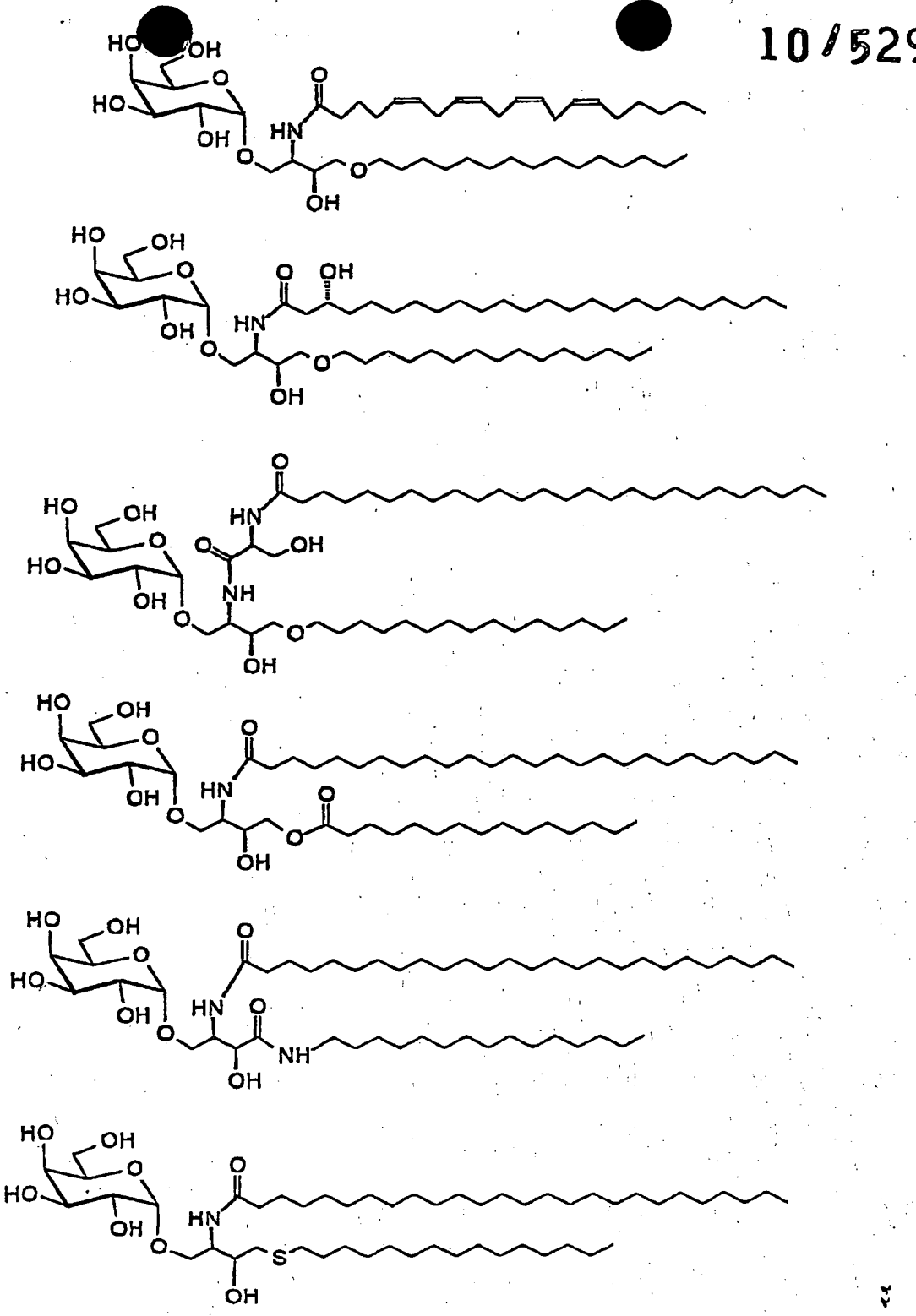


FIG. 8 α-GalCer analogues with modified sphingosine

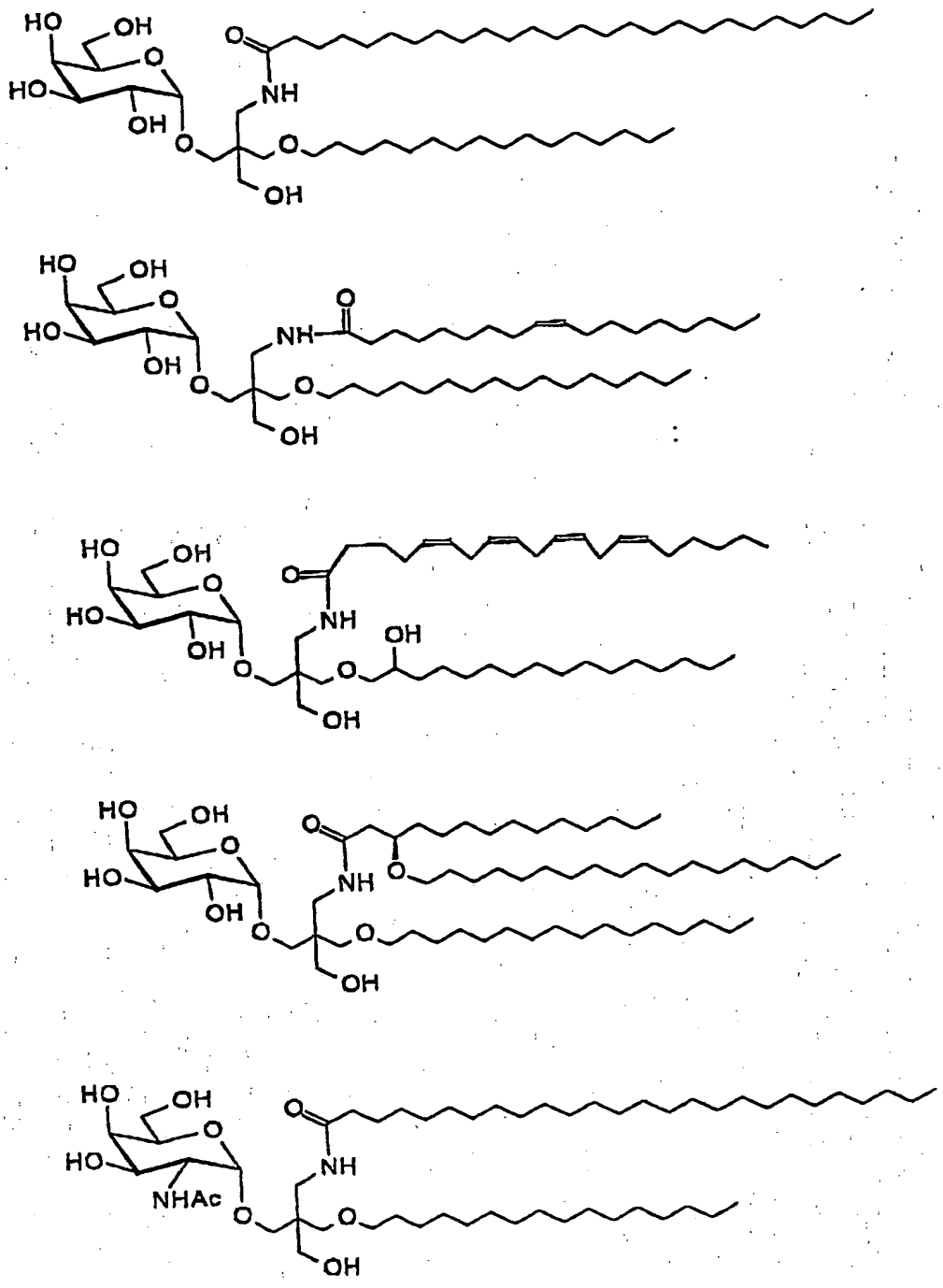


FIG. 9 α-GalCer analogues based on pentaerythritol

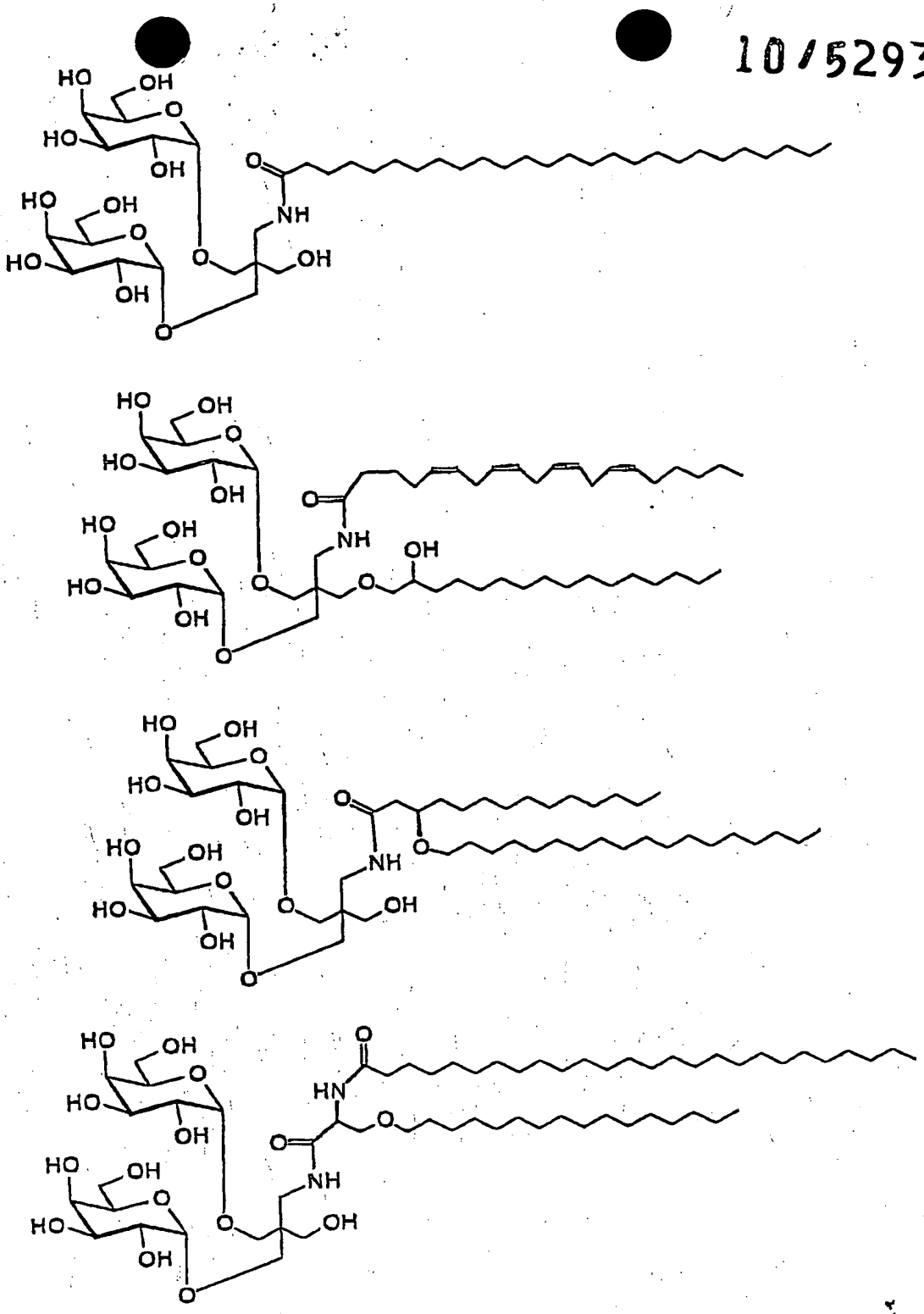


FIG. 10 Divalent α-GalCer analogues based on pentaerythritol

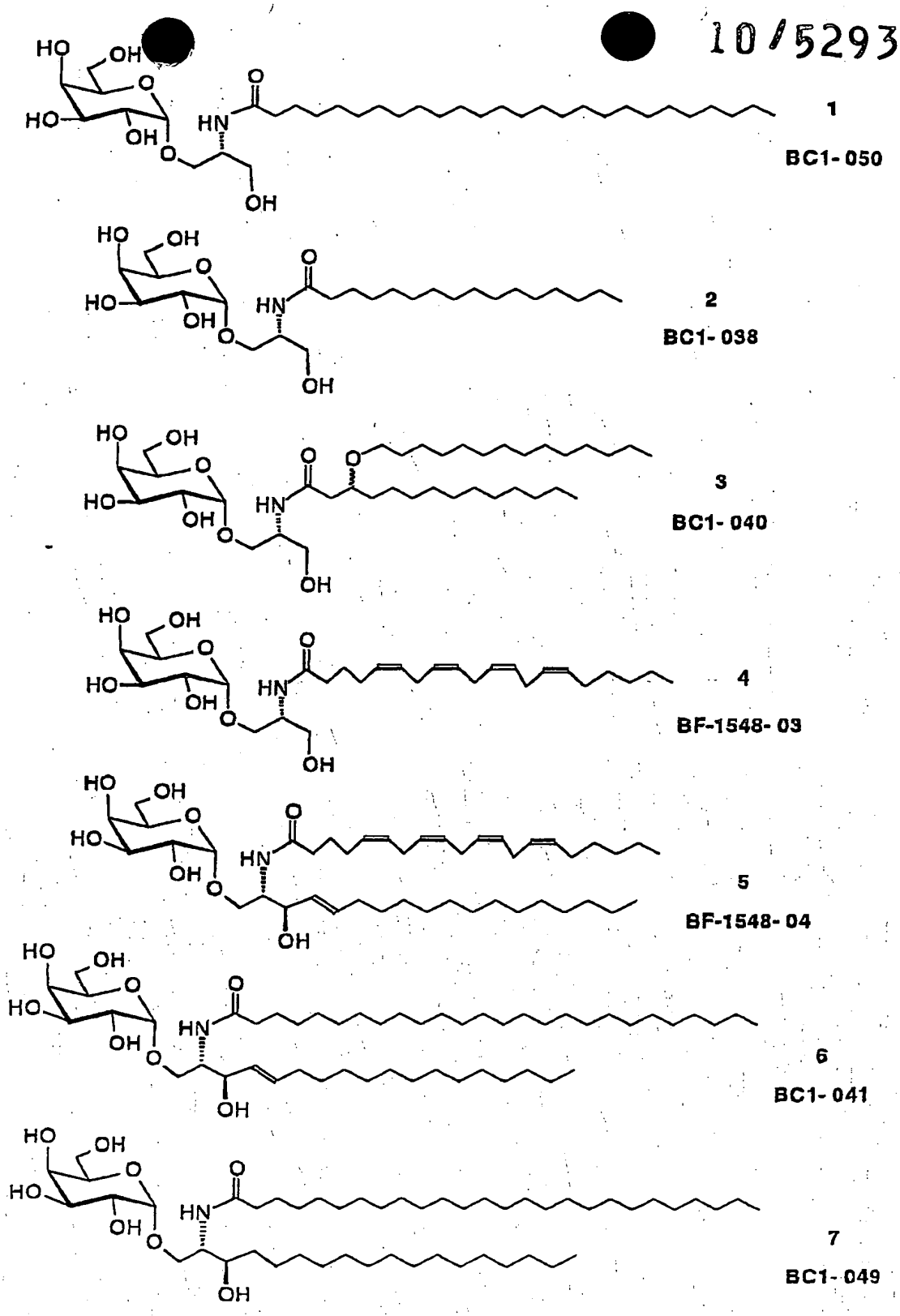
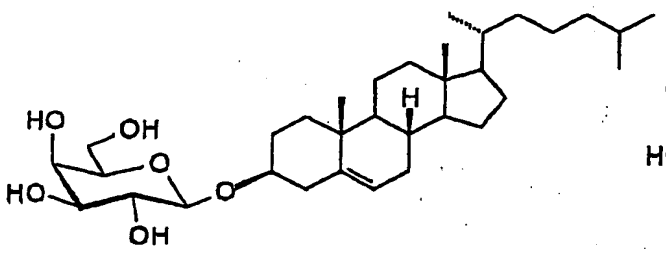
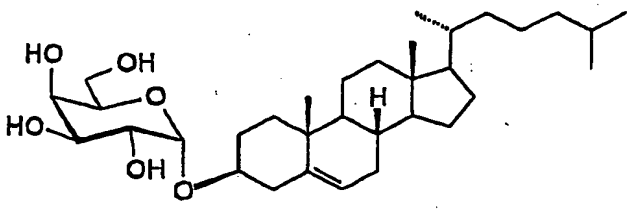


FIG. 11 α-GalCer analogues (1 - 7) prepared in this invention disclosure



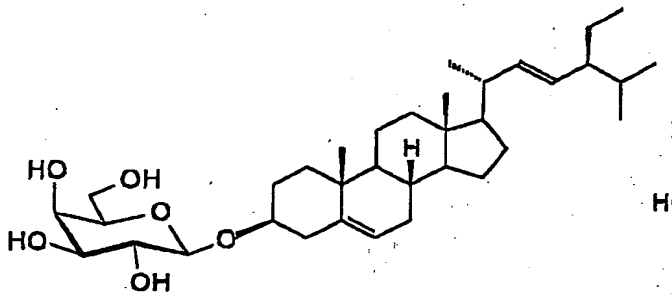
8

BC 1-046



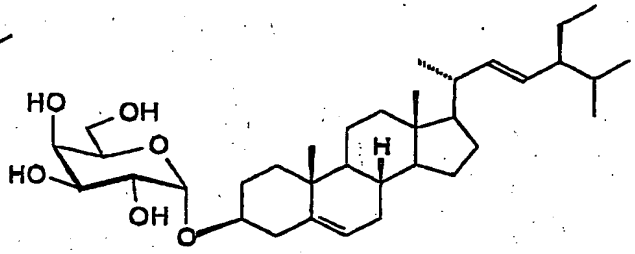
9

BC 1 - 051



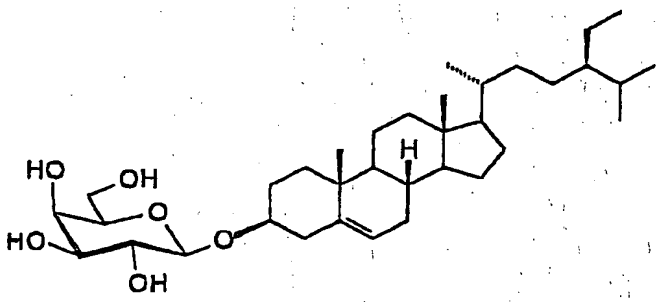
10

BC 1-048



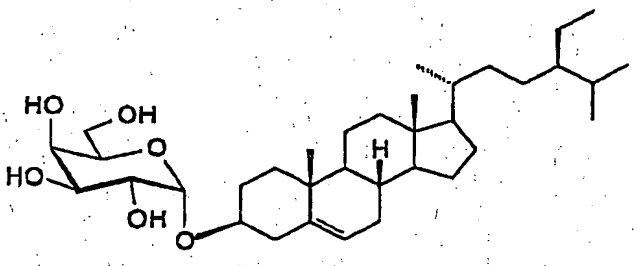
11

BC 1-047



12

BC 1-054



13

FIG. 12 Steroidal galactosides (8 - 13) prepared in this invention disclosure

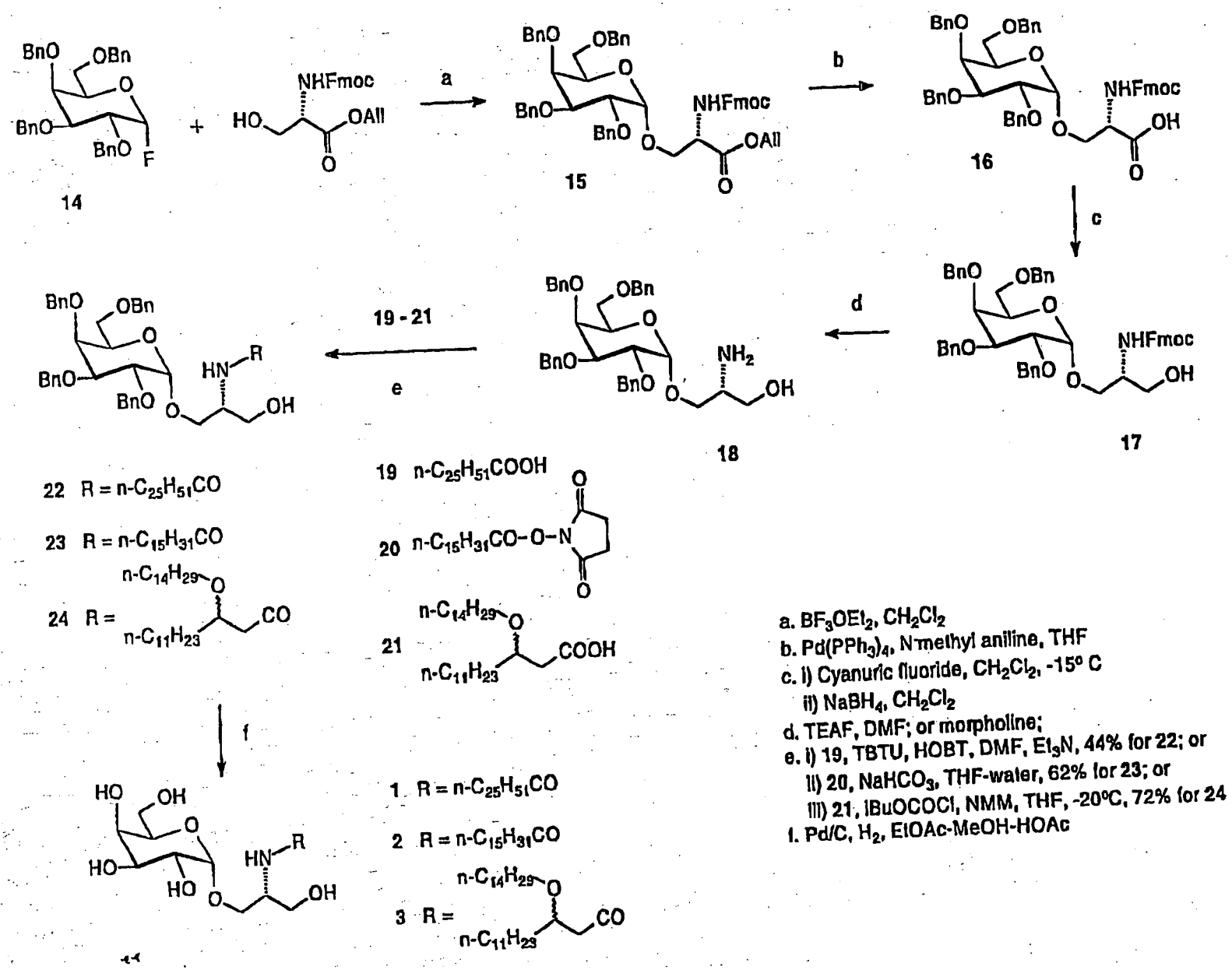


FIG. 13 Preparation of α-GalCer analogues 1 - 3

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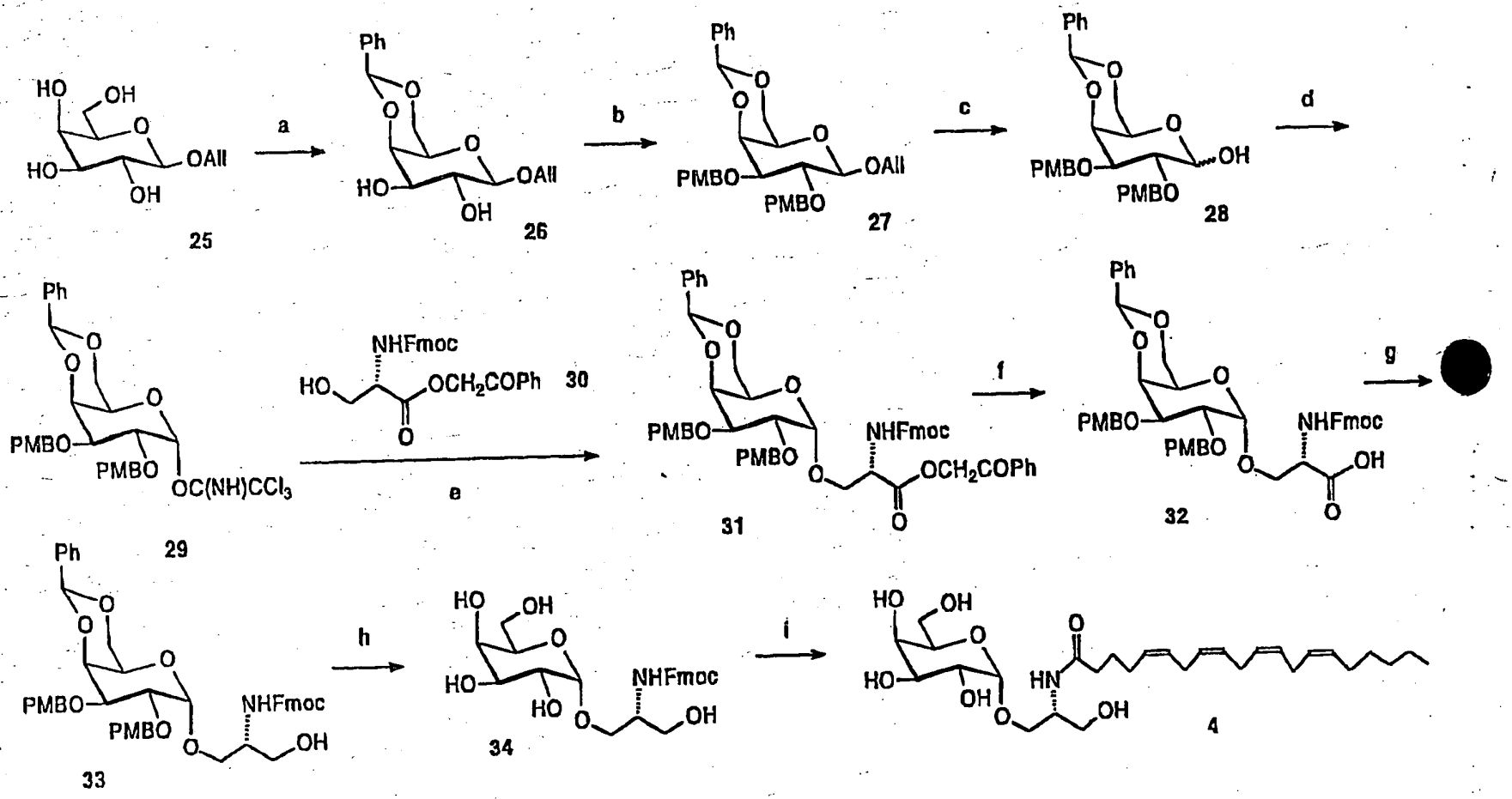

a. $PhCH(OMe)_2$, p-TsOH, CH_3CN
b. p-MeO-$PhCH_2Cl$, NaH, DMF
c. i) [bis(methyldipheylphosphine)](1,5-cyclooctadiene) iridium(I) hexafluorophosphate, THF; ii) NBS, THF-H_2O
d. $CNCCl_3$, DBU, CH_2Cl_2
e. TMSOTf, THF
f. Zn, HOAc
g. i) Cyanuric fluoride, CH_2Cl_2, -15° C; ii) $NaBH_4$, CH_2Cl_2
h. TFA, CH_2Cl_2
i. i) morpholine, rt; ii) 36, acetone-H_2O,rt, 75%
j. NHS, DCC, EtOAc, rt, 61%

FIG. 14 Preparation of α-GalCer analogue 4

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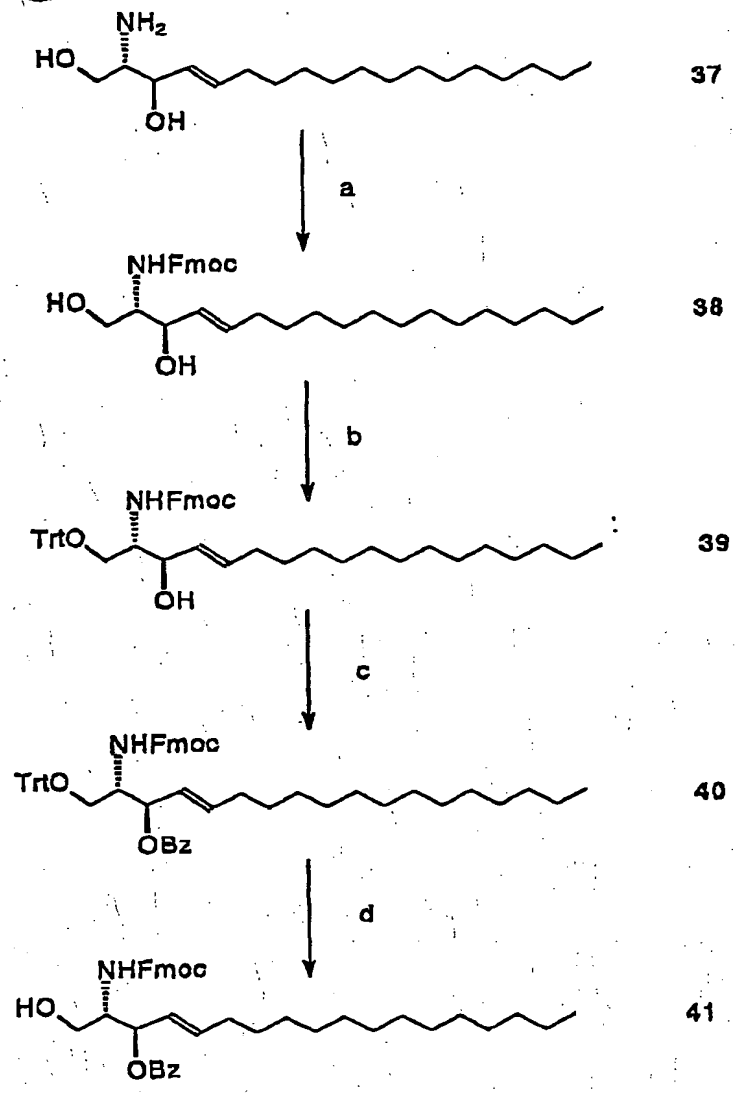


a. Fmoc-N-hydroxy succinimide, $NaHCO_3$, acetone-H_2O
b. Trt-Cl, Py, DMAP
c. BzCl, Py, DMAP
d. P-TsOH, MeOH-CH_2Cl_2

FIG. 15 Preparation of *E*-4,5-ene-sphingosine acceptor 41

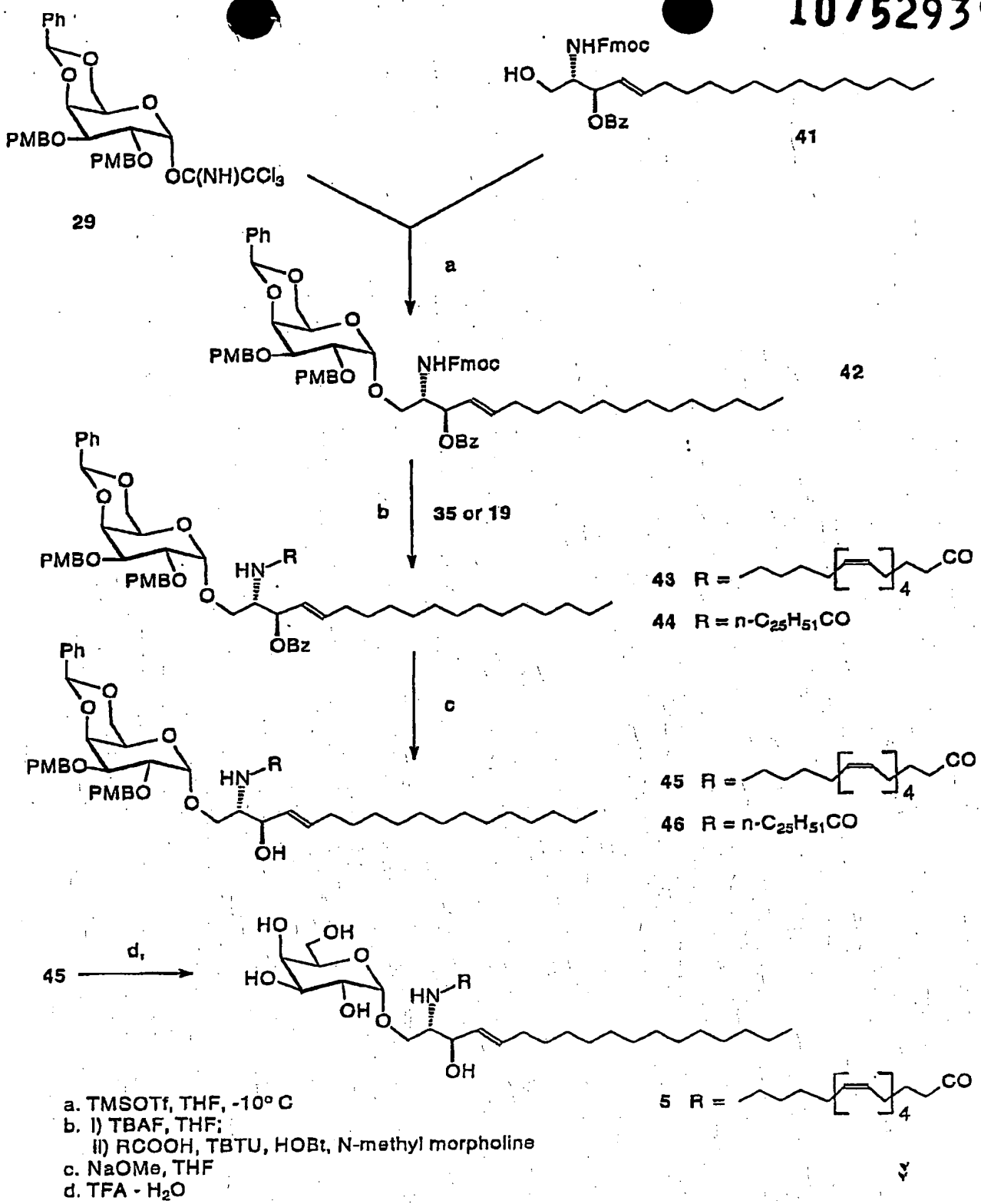


FIG. 16 Preparation of α-GalCer analogue 5

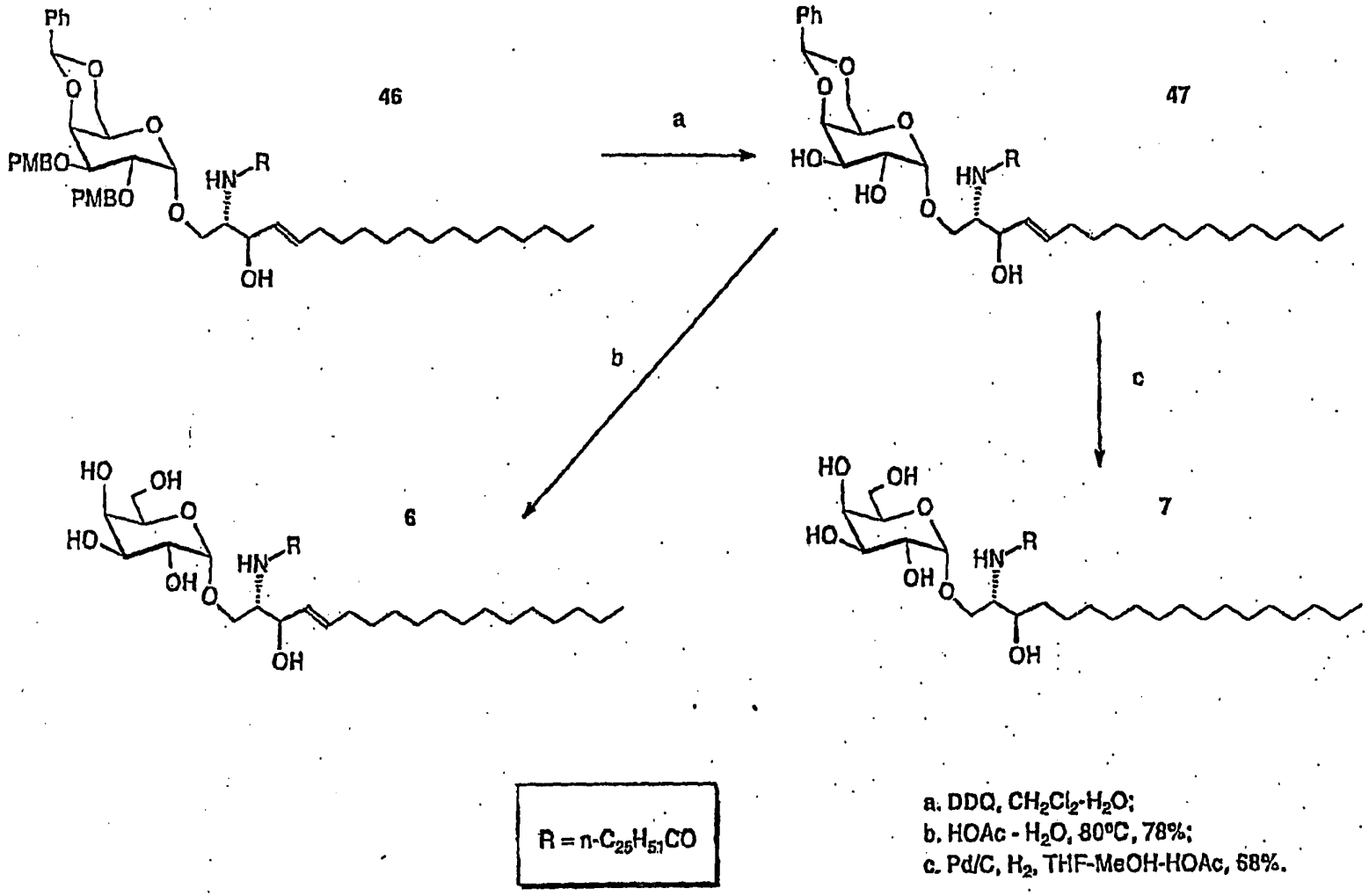
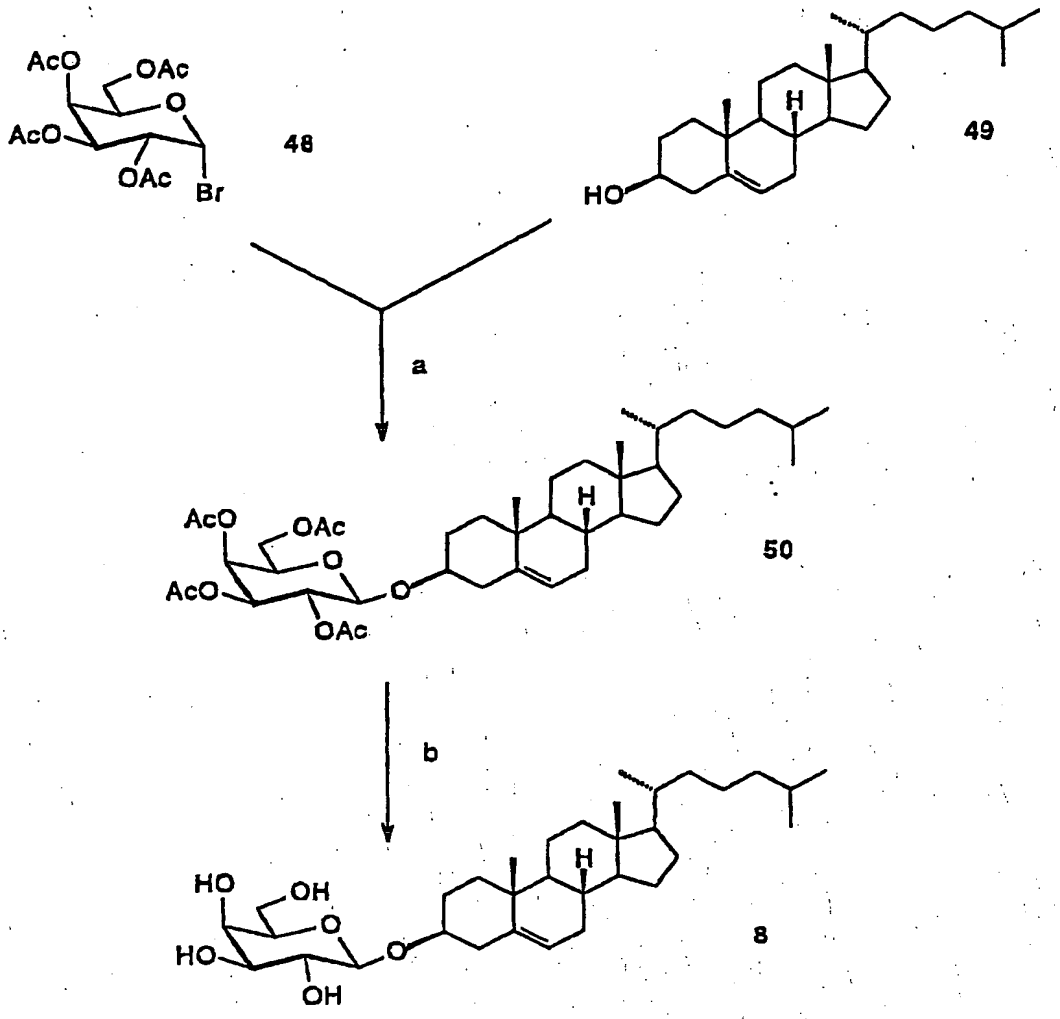


FIG. 17 Preparation of α-GalCer analogues 6 and 7

10/529393



a. $Hg(CN)_2$, $HgBr_2$, CasO4, CH3CN - C6H6, rt, 69%;
b. 0.1 M NaOMe, $CHCl_3$, rt, 83%.

FIG. 18 Preparation of steroidal glycoside 8

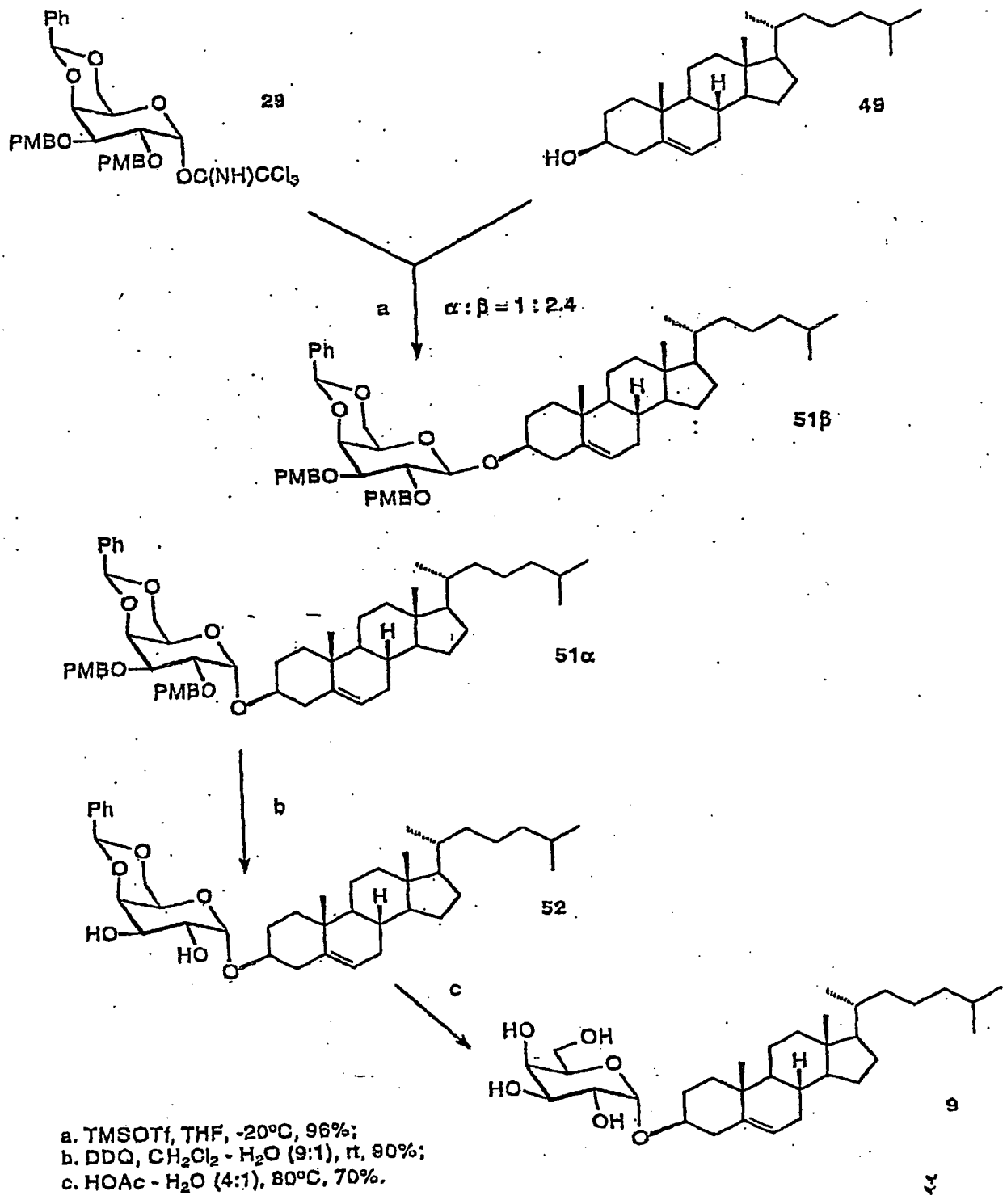
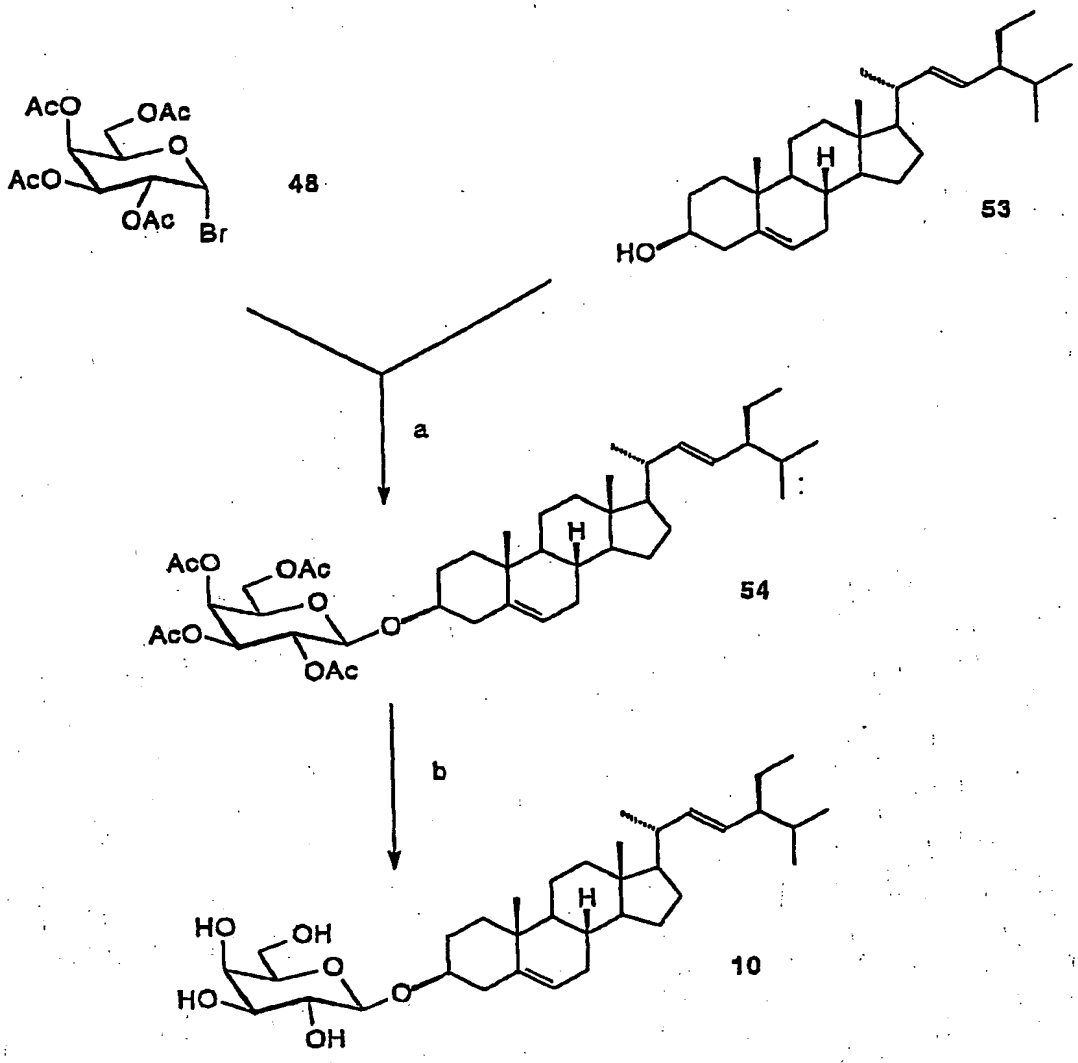
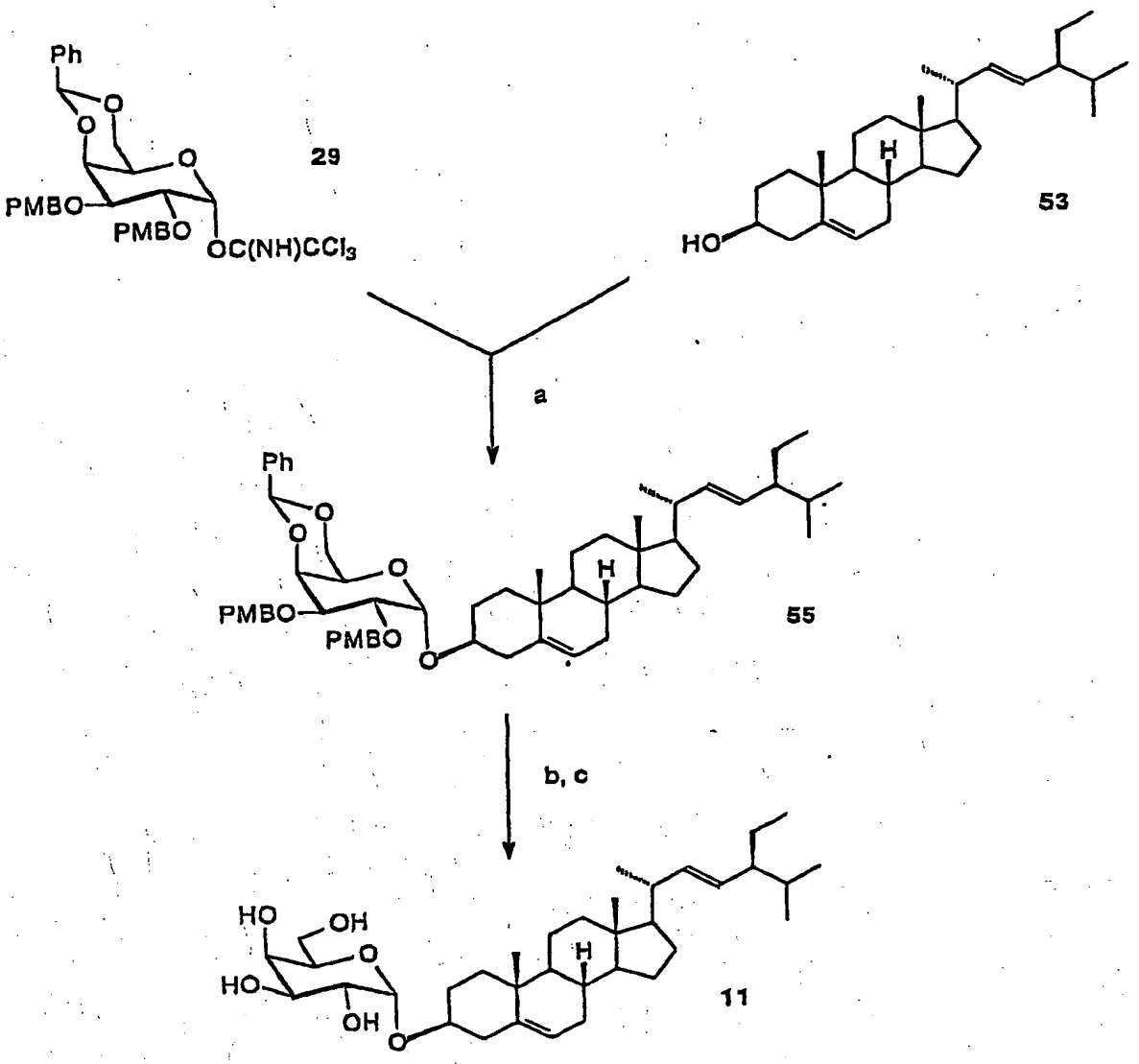


FIG. 19 Preparation of steroidal glycoside 9



a. $Hg(CN)_2$, $HgBr_2$, $CasO_4$, CH_3CN - C_6H_6, rt, 70%;
b. 0.1 M NaOMe, $CHCl_3$, rt, 58%.

FIG. 20 Preparation of steroidal glycoside 10



a. TMSOTf, THF, -20°C, 31%;
b. DDQ, CH_2Cl_2 - H_2O (9:1), rt, 76%;
c. HOAc - H_2O (4:1), 80°C, 63%.

FIG. 21 Preparation of steroidal glycoside 11

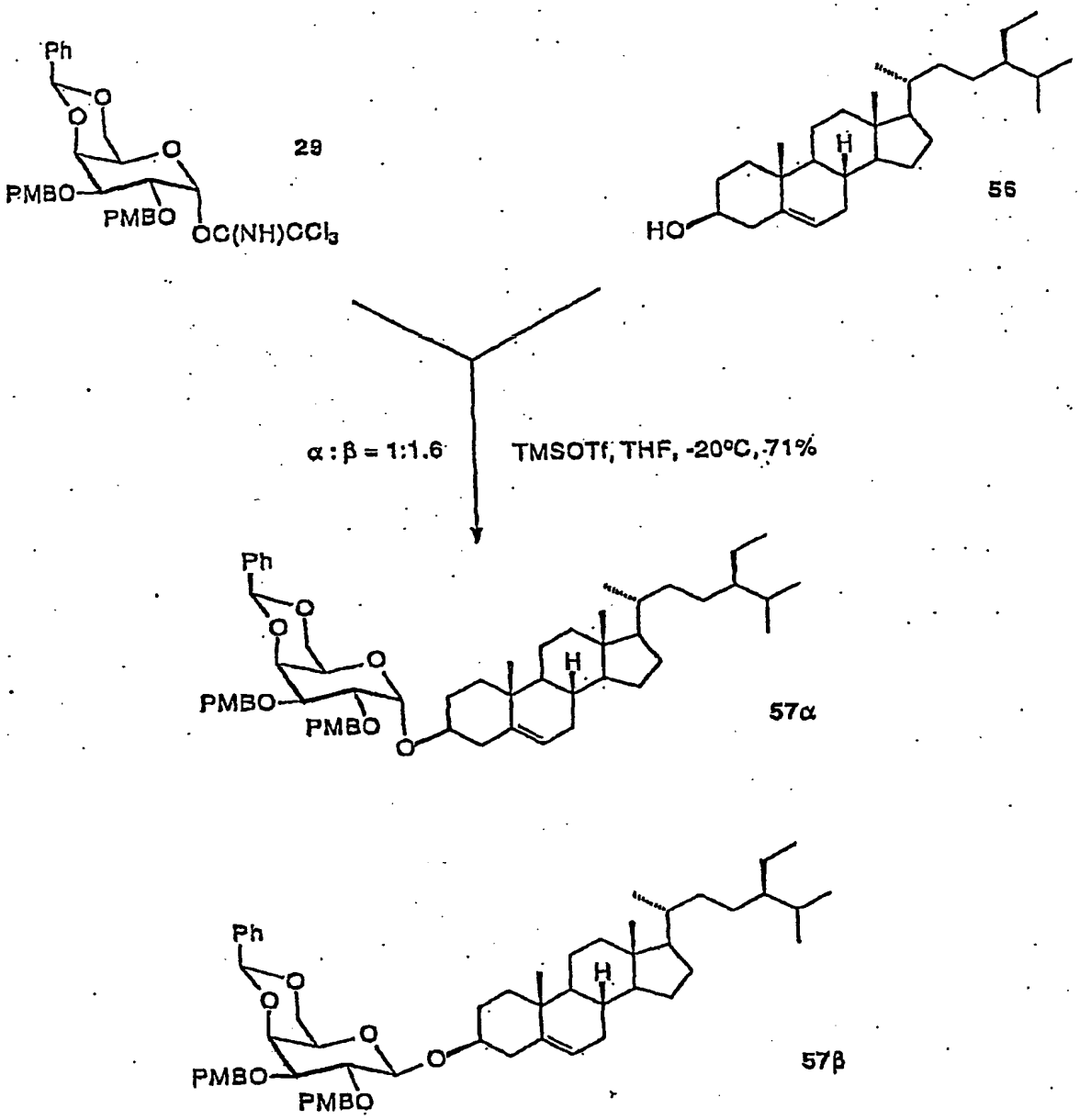
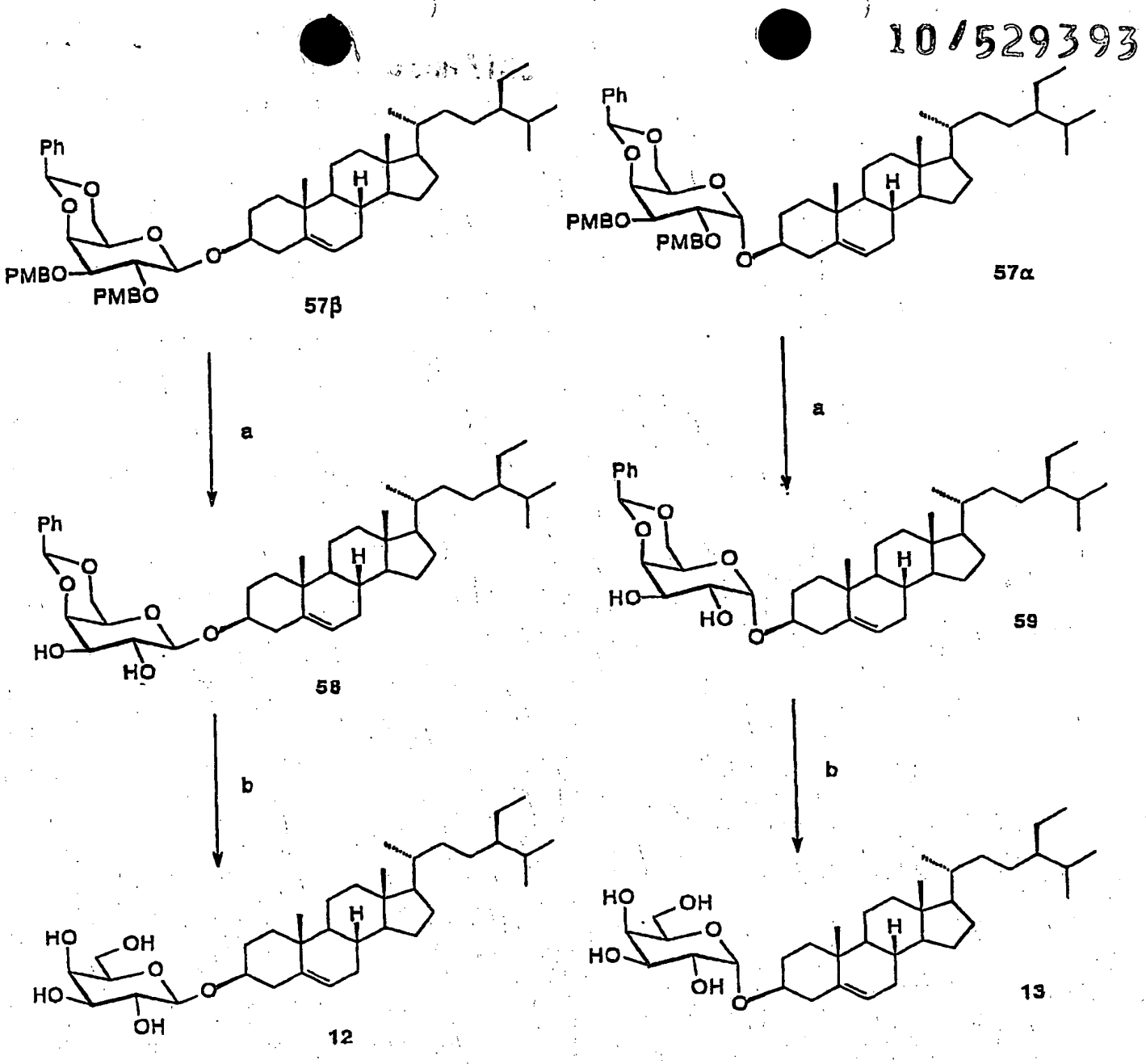


FIG. 22 Preparation of steroidal glycosides 57α and 57β

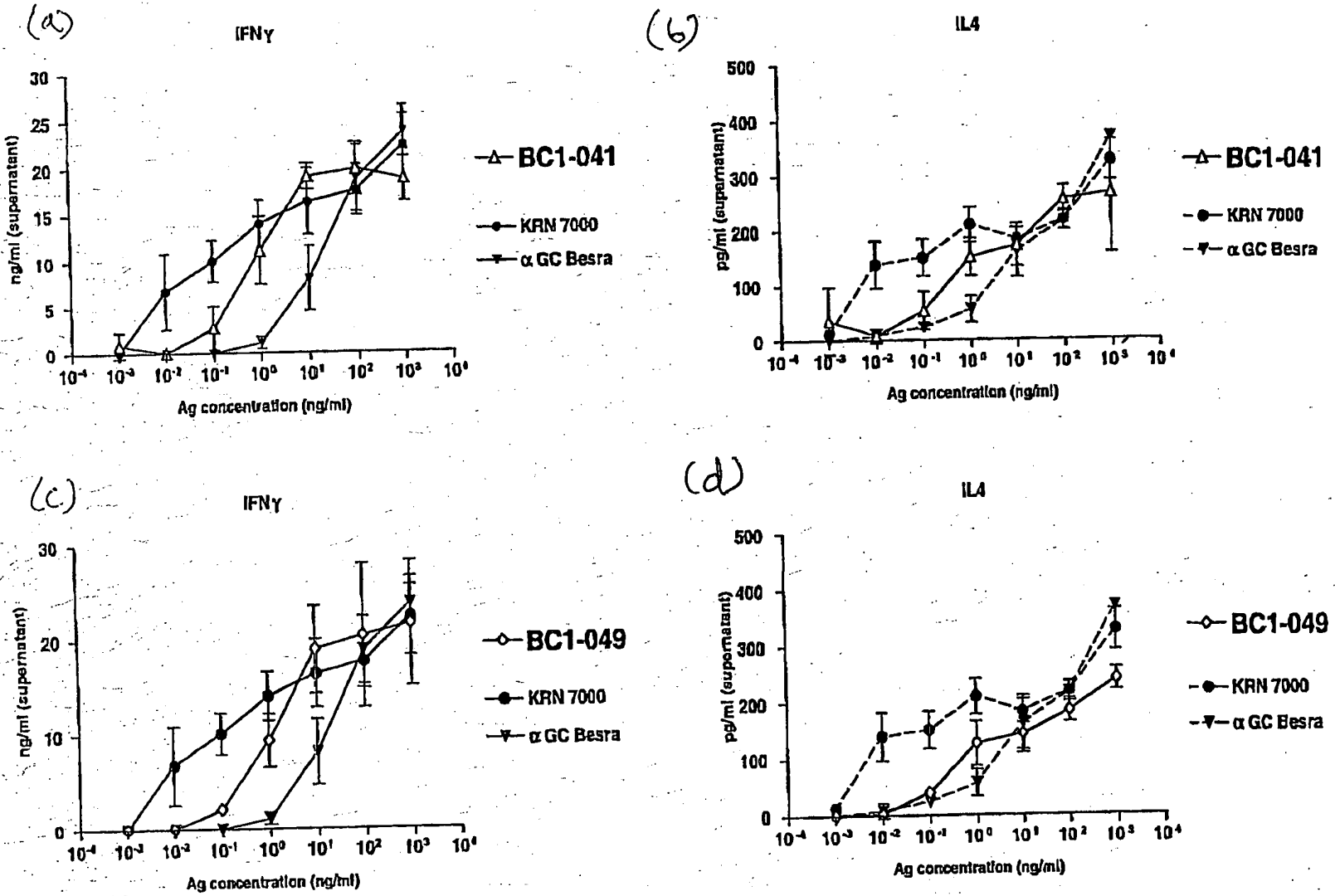


a. DDQ, CH_2Cl_2 - H_2O (9:1), rt, 73% for 58 and 77% for 59;
b. HOAc - H_2O (4:1), 80°C, 73% for 12 and 60% for 13.

FIG. 23 Preparation of steroidal glycosides 12 and 13

FIG 24

Cytokine Secretion (ELISA: BALB/c Speen)



10/529393

Fig. 25 (a)

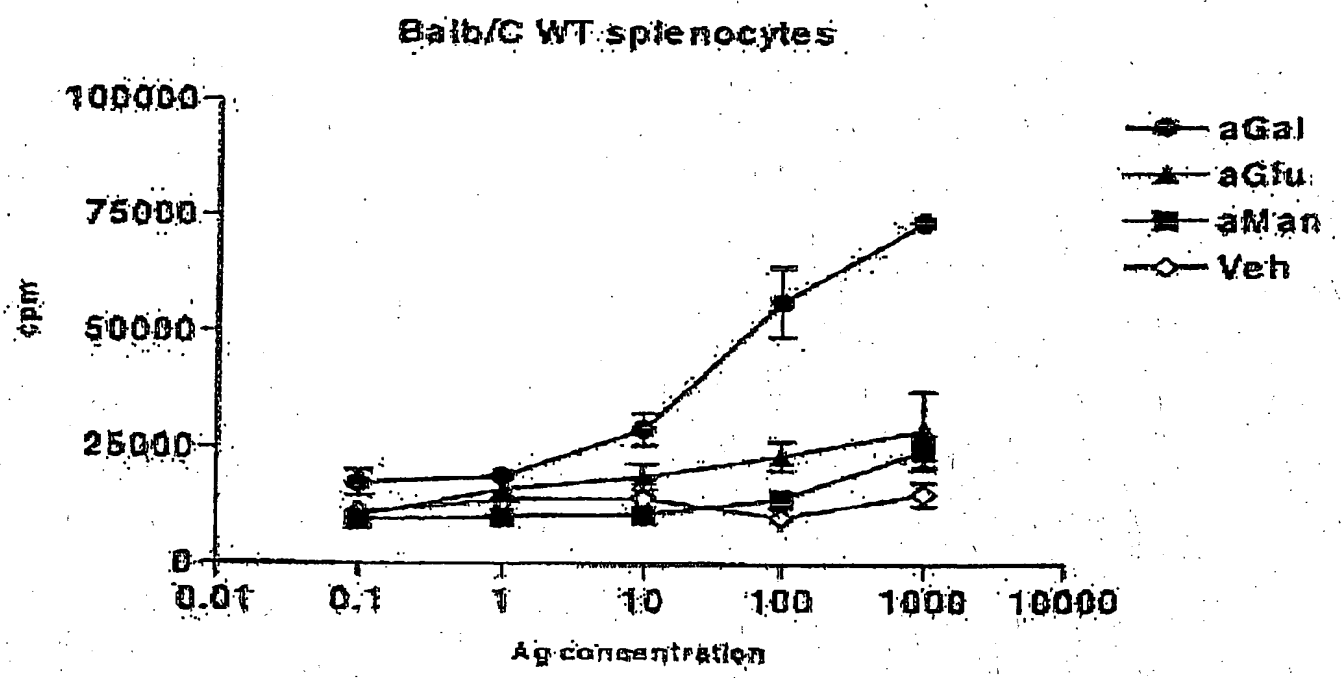

Balb/C WT splenocytes
100000
75000
50000
25000
0
cpm
0.01
0.1
1
10
100
1000
10000
Ag concentration
aGal
aGfu
aMan
Veh

Fig. 25 (b)

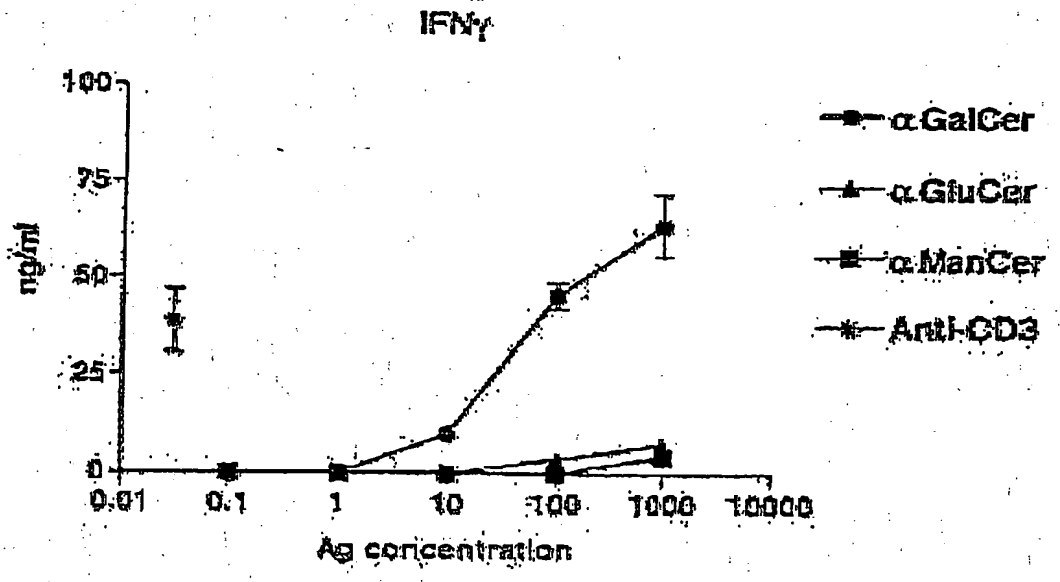

IFNγ
100
75
50
25
0
ng/ml
0.01
0.1
1
10
100
1000
10000
Ag concentration
αGalCer
αGluCer
αManCer
Anti-CD3

Fig. 25(c)

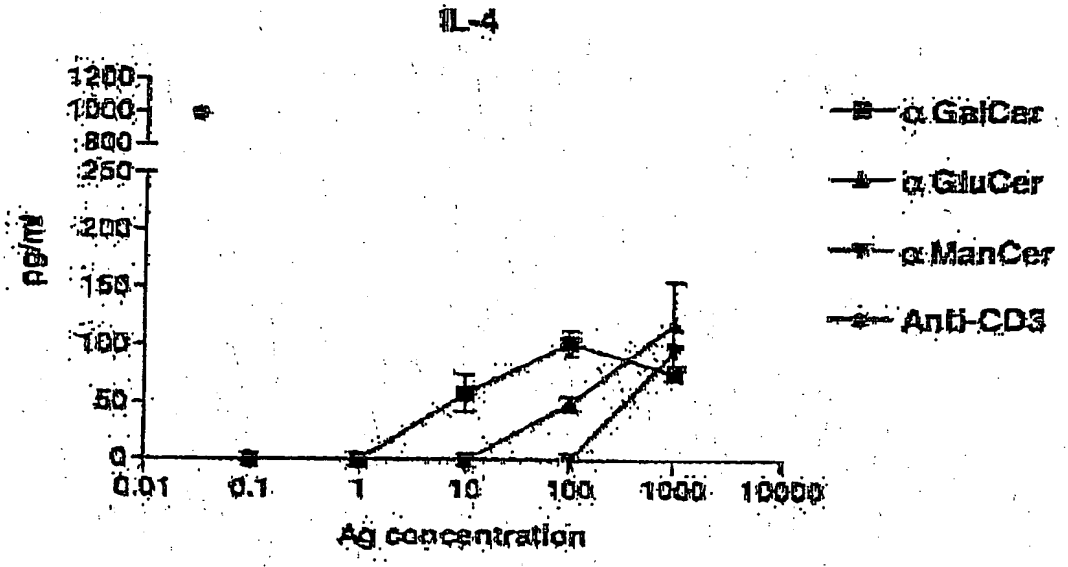

IL-4
1200
1000
800
250
200
150
100
50
0
pg/ml
0.01
0.1
1
10
100
1000
10000
Ag concentration
α GalCer
α GluCer
α ManCer
Anti-CD3

Fig. 25 (d)

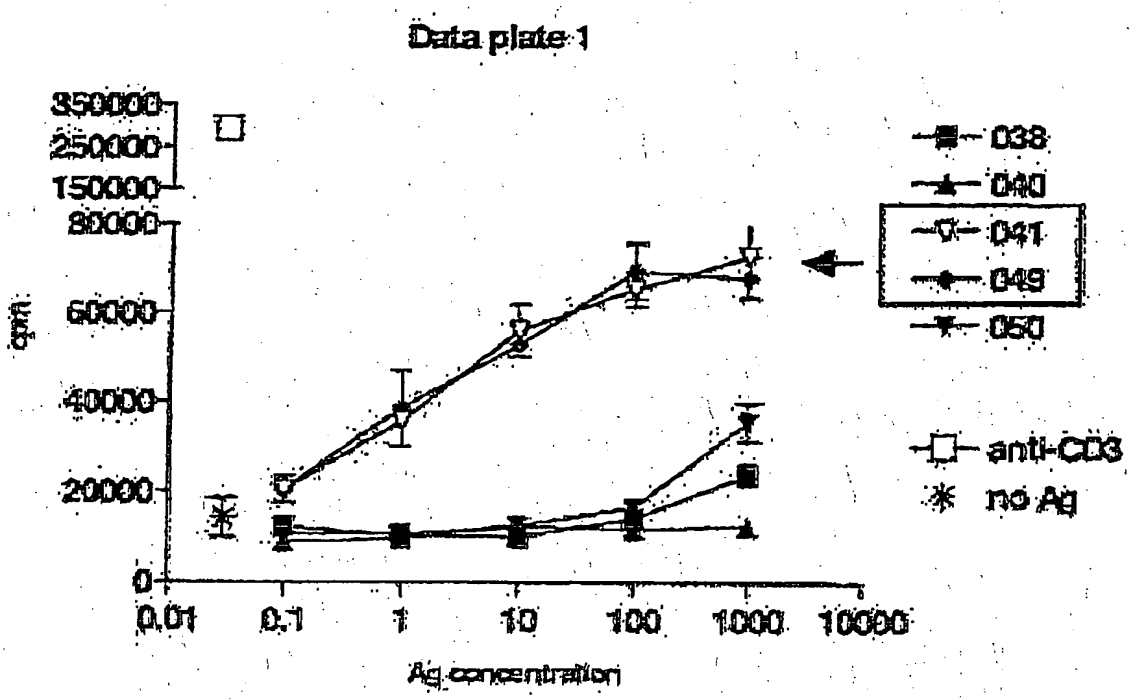

Data plate 1
350000
250000
150000
80000
60000
40000
20000
0
cpm
0.01
0.1
1
10
100
1000
10000
Ag concentration
038
040
041
049
050
anti-CD3
no Ag

Fig 25 (c)

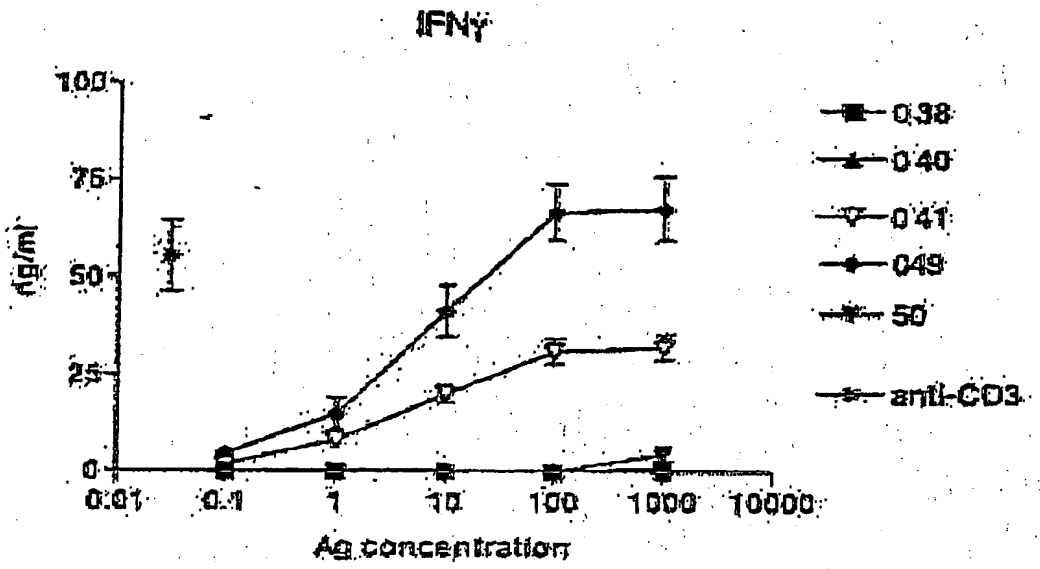

IFNγ
100
75
50
25
0
(pg/m)
0.01
0.1
1
10
100
1000
10000
Ag concentration
038
040
041
049
50
anti-CD3

Fig. 25 (4)

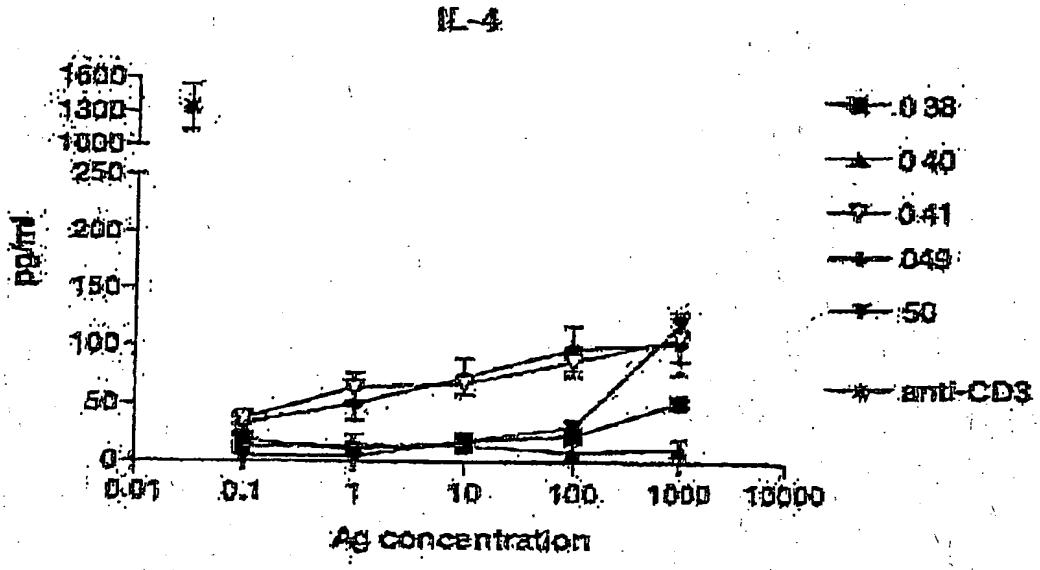

IL-4
pg/ml
1600
1300
1000
250
200
150
100
50
0
0.01
0.1
1
10
100
1000
10000
Ag concentration
0 38
0 40
0 41
049
50
anti-CD3

Fig. 25 (h)

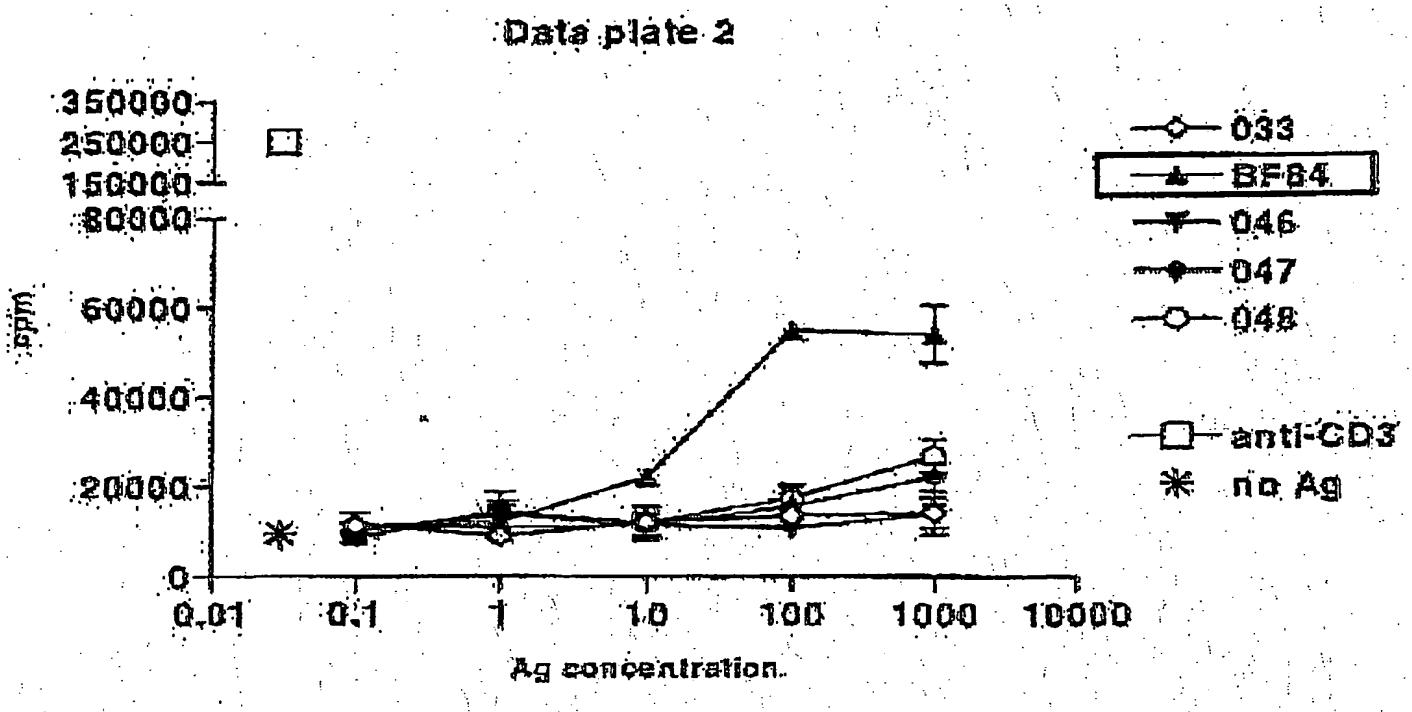

Data plate 2
350000
250000
150000
80000
60000
40000
20000
0
cpm
0.01
0.1
1
10
100
1000
10000
Ag concentration
033
BF84
046
047
048
anti-CD3
no Ag

Fig 25 (D)

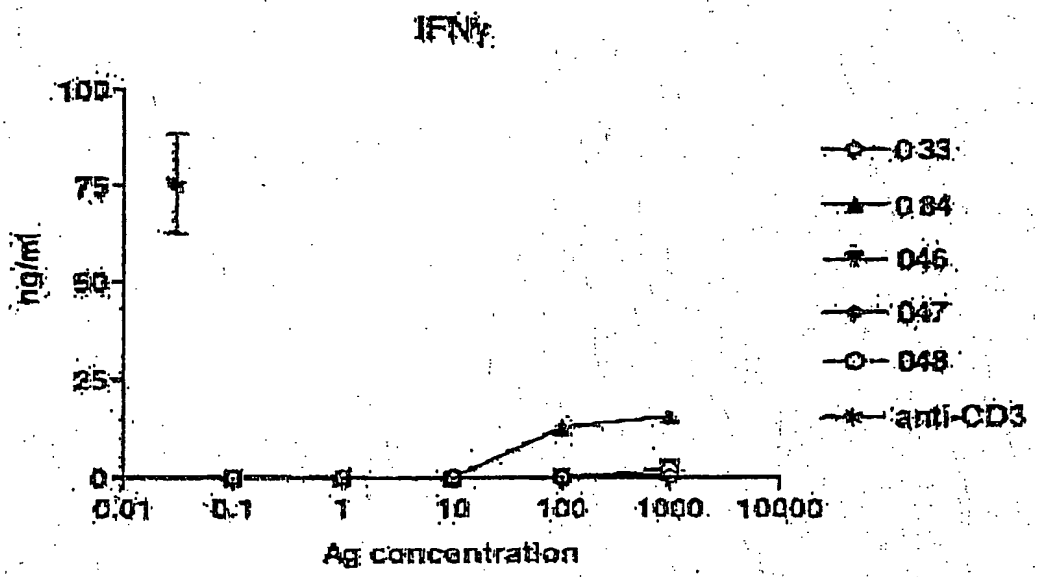

IFNγ
100
75
50
25
0
ng/ml
0.01
0.1
1
10
100
1000
10000
Ag concentration
033
034
046
047
048
anti-CD3

Fig. 25 (j)

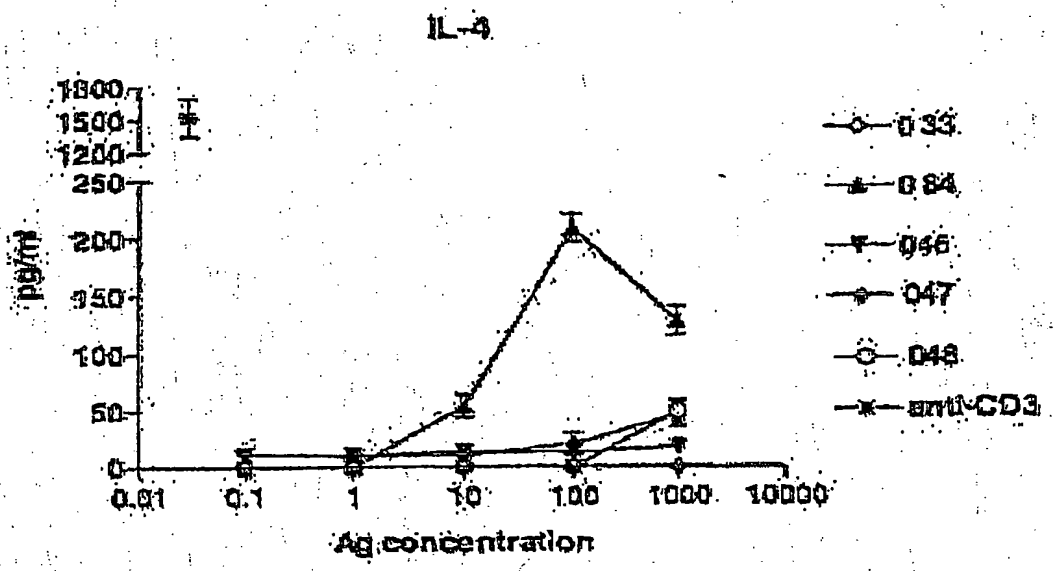
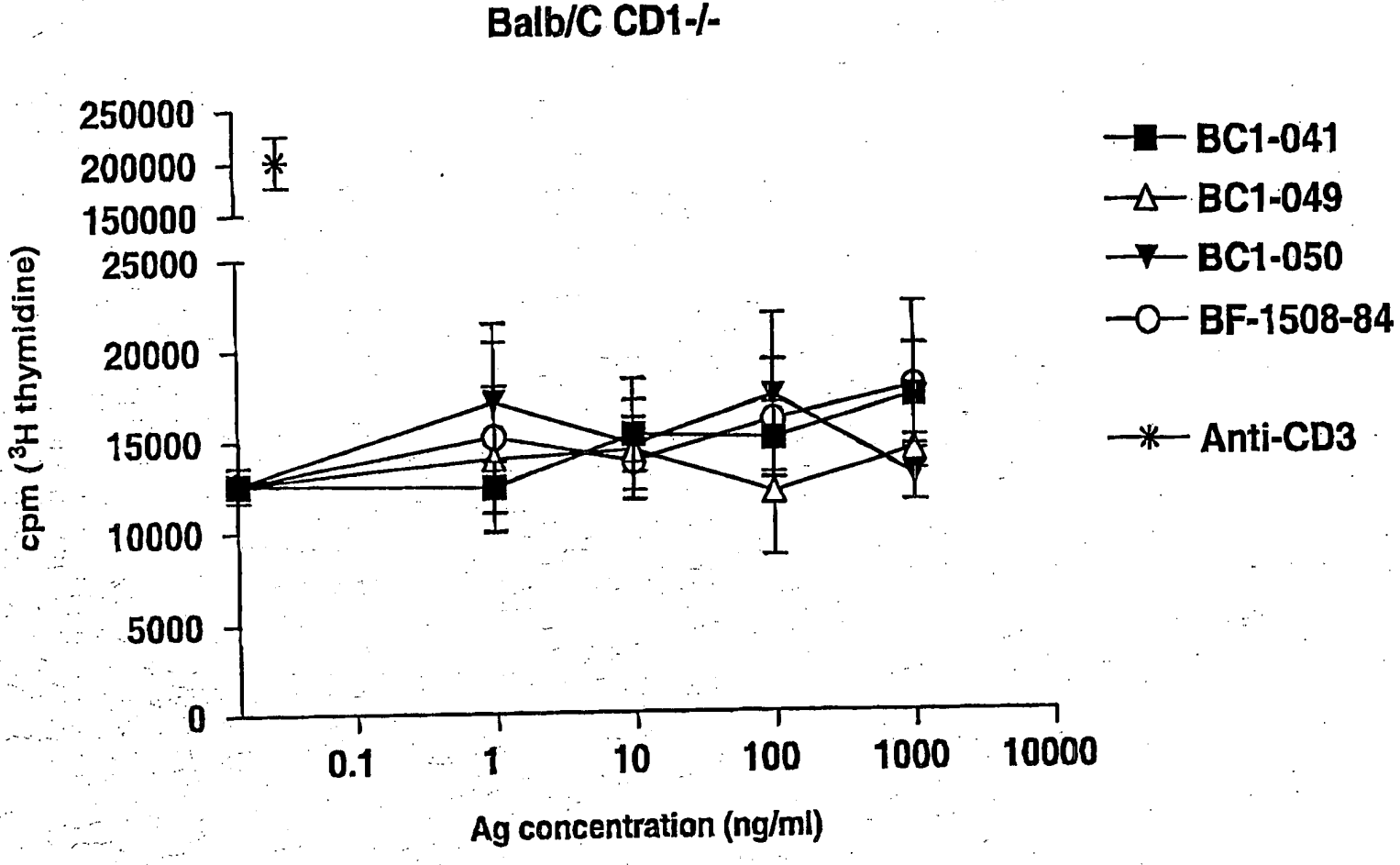

IL-4
1800
1500
1200
250
200
150
100
50
0
pg/ml
0.01
0.1
1
10
100
1000
10000
Ag concentration
033
034
046
047
048
anti-CD3

FIG 26



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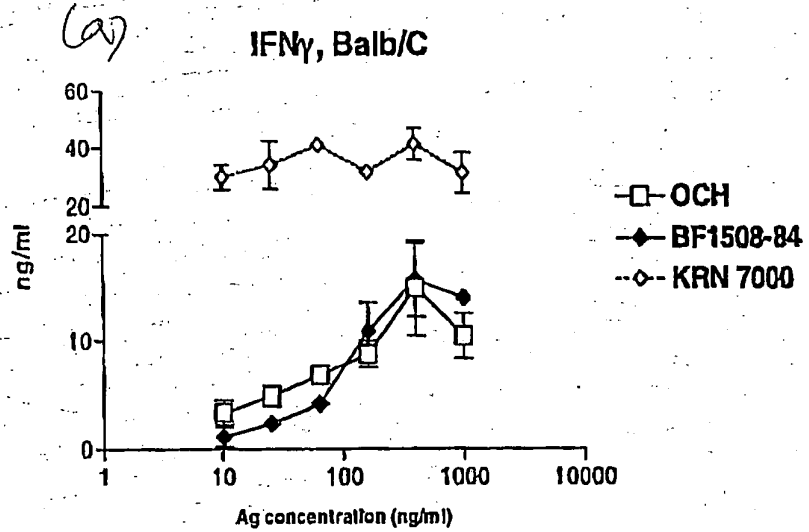
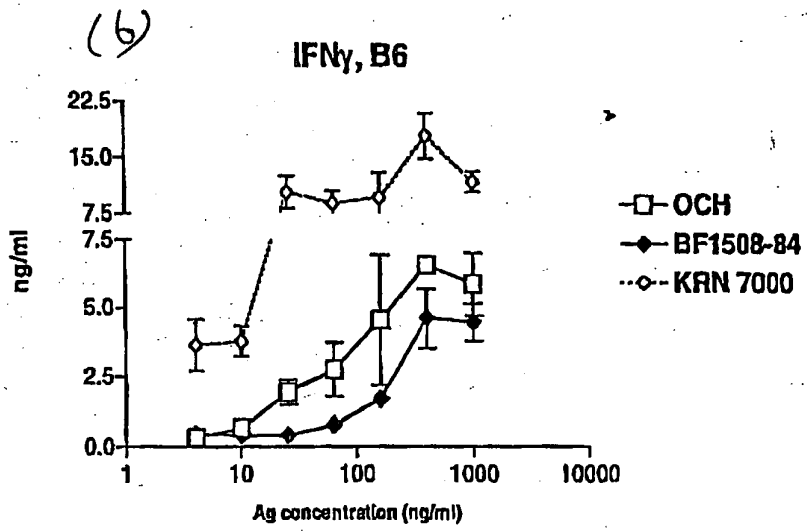
Cytokine production:

FIG 27

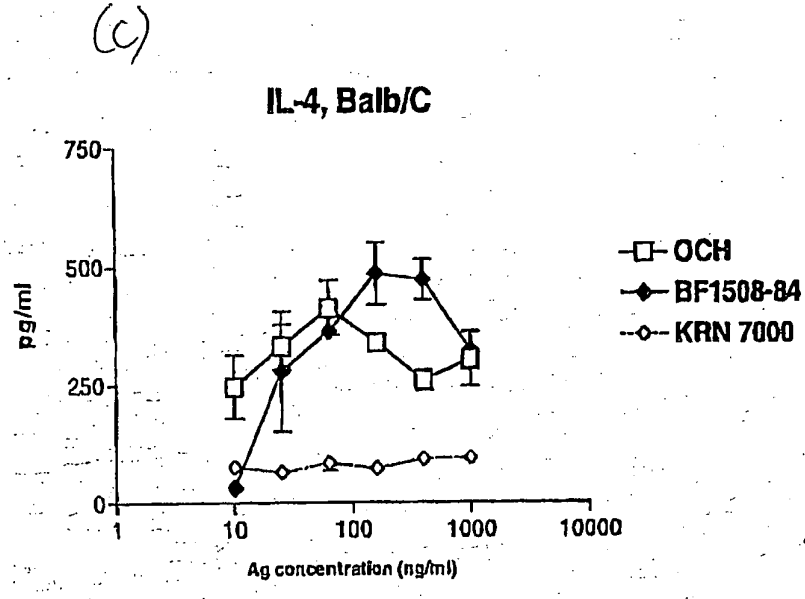
(a)



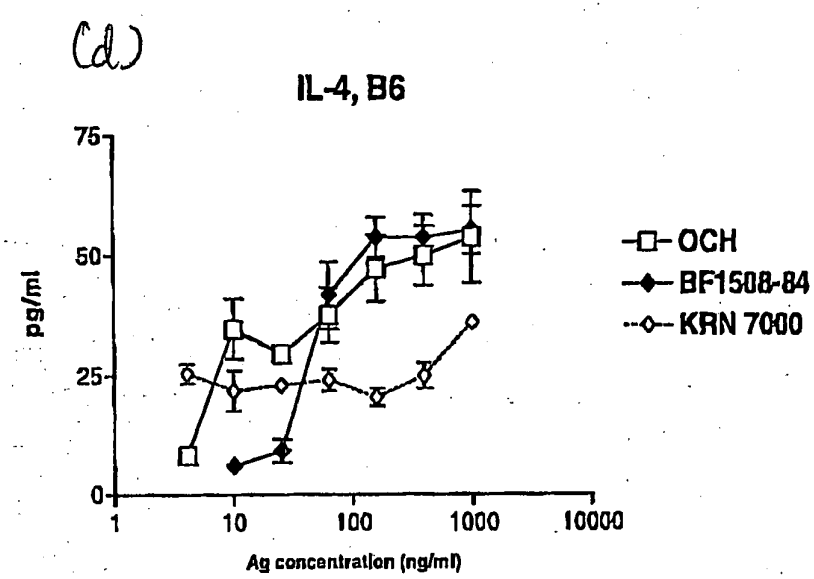
(b)



(c)



(d)

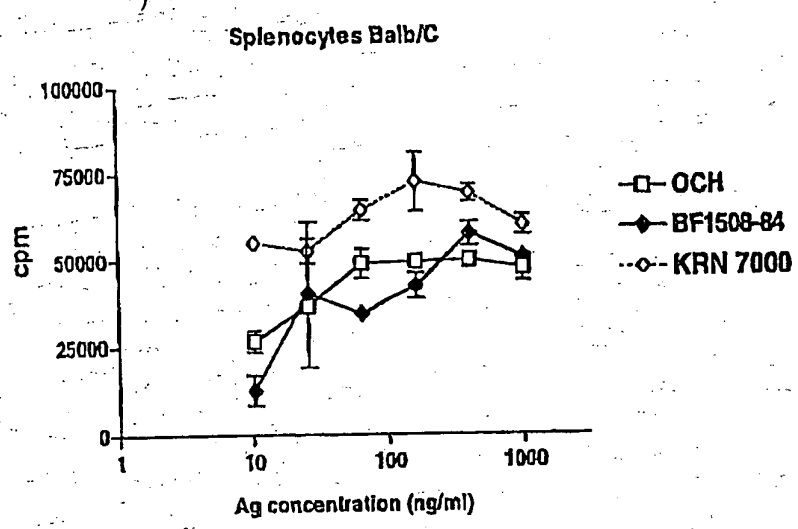


10/529392

FIG 28

Proliferation:

(a)



(b)

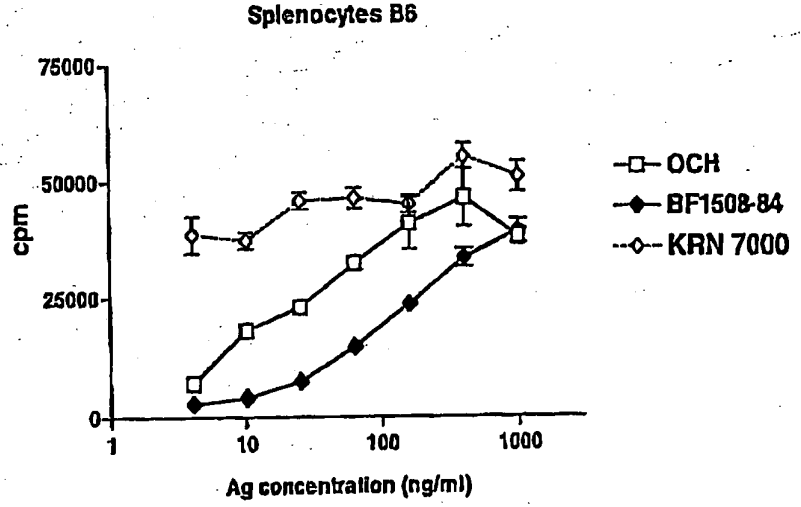


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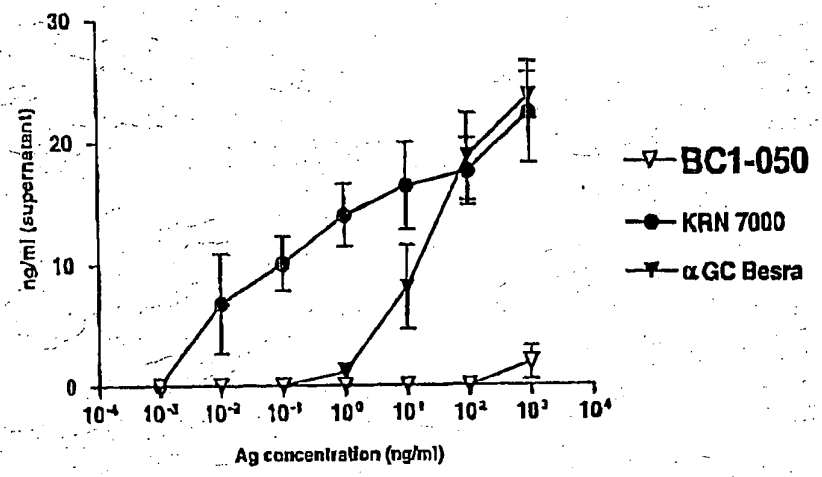
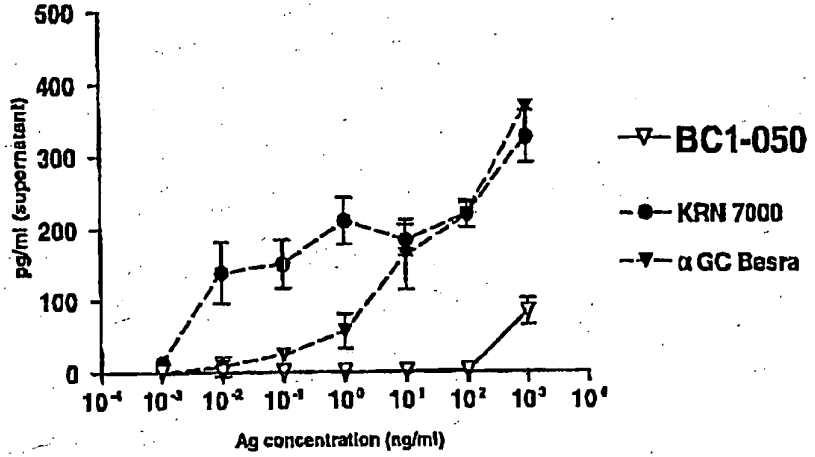
FIG 29

Cytokine Secretion (ELISA: BALB/c Speen)

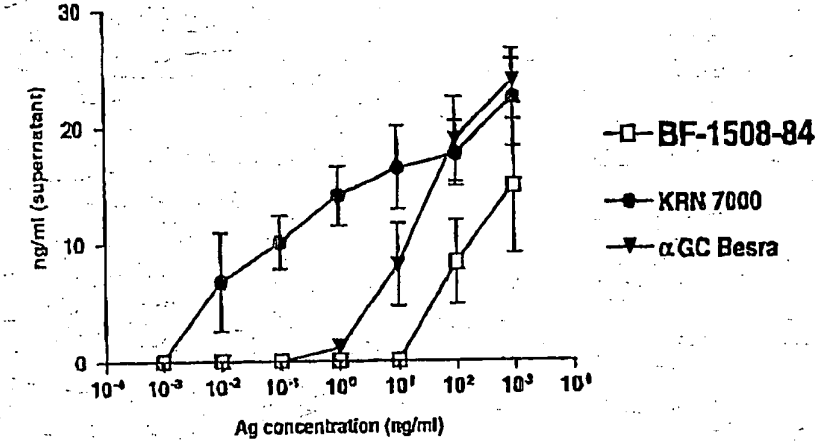
(a)



(b)



(c)



(d)

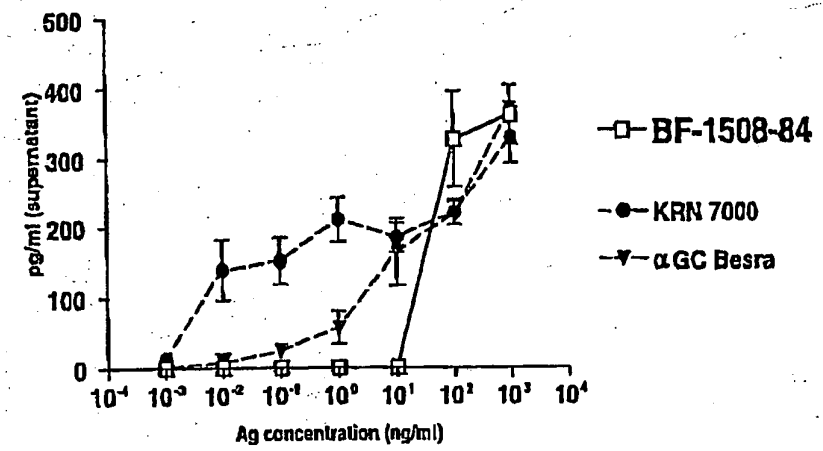


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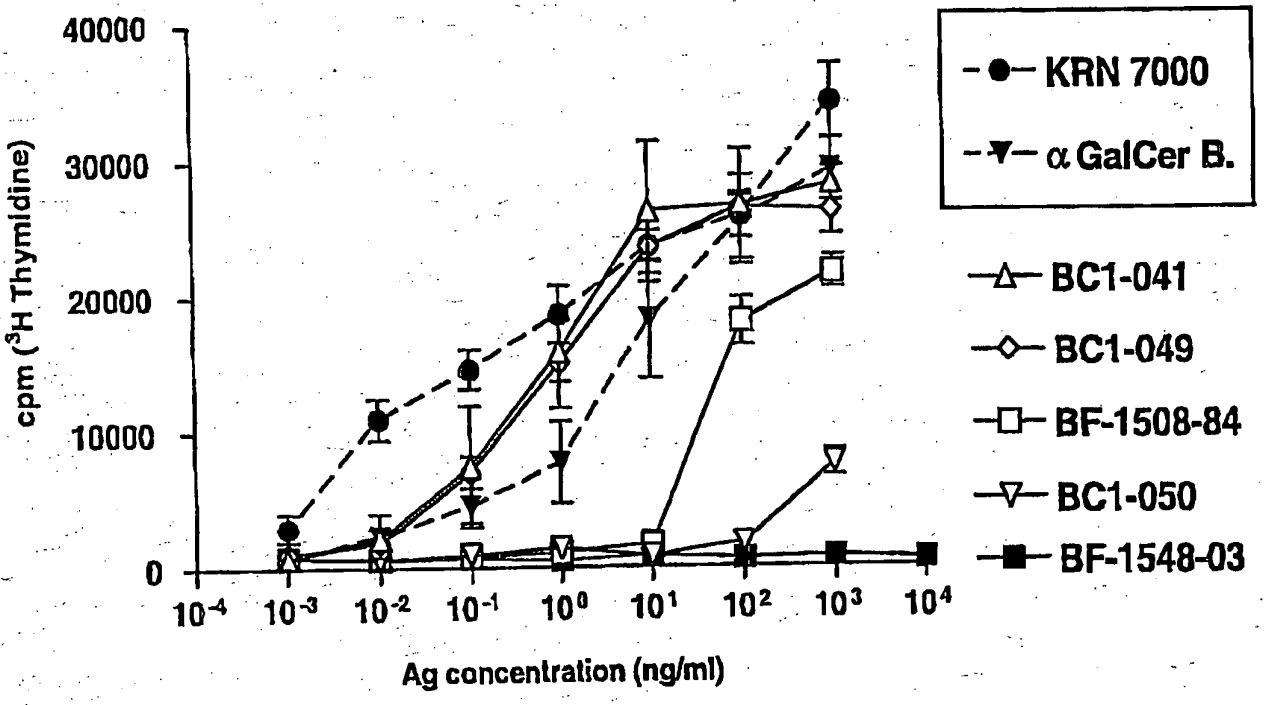
Fig. 30

Proliferation (BALB/c Spleen)



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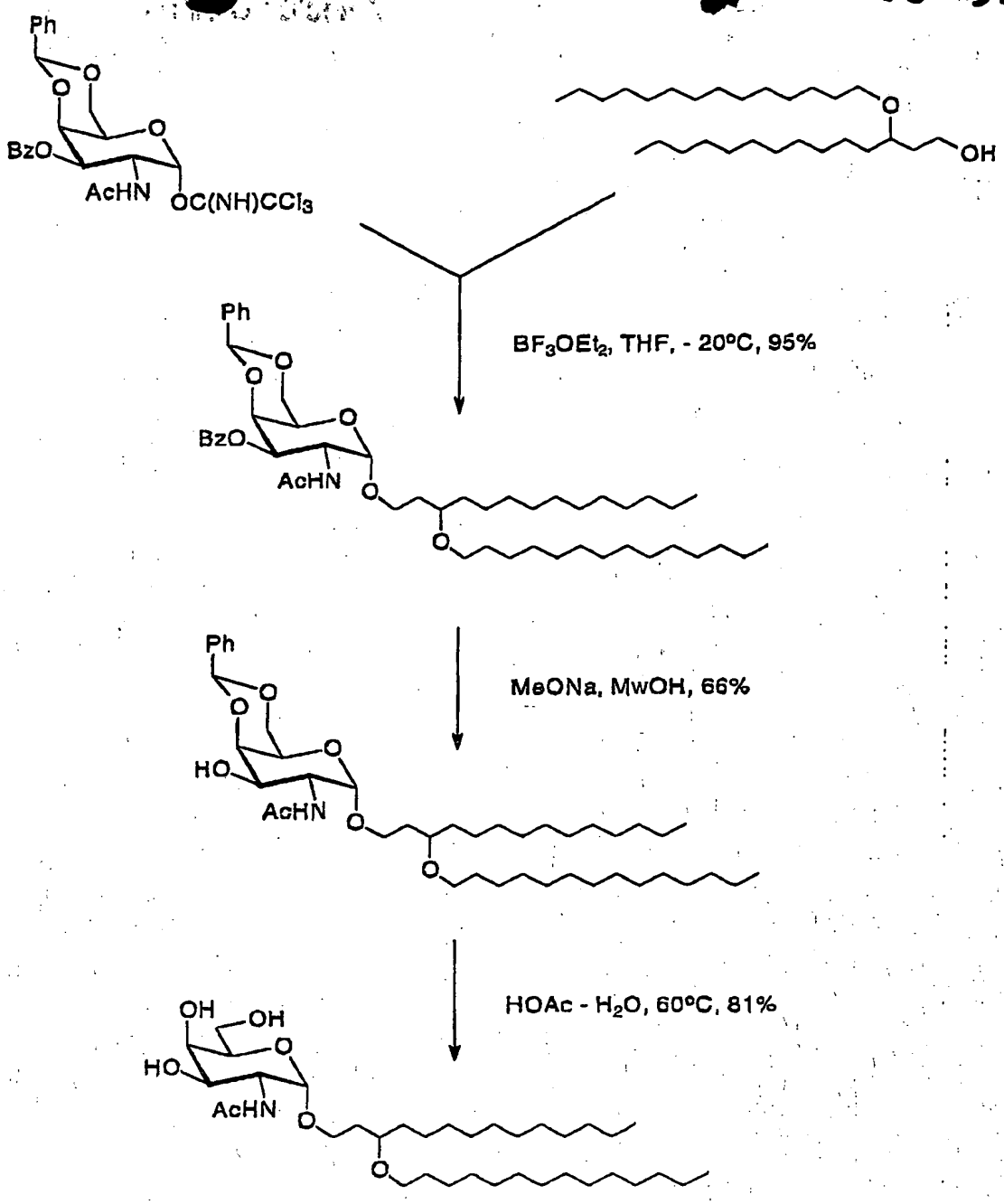

Preparation of glycolipid **033** (**BC1-033**)

FIG. 31